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SYMBIOTIC EFFECTIVENESS IN NODULATED RED CLOVER

III. FURTHER STUDIES ON INHERITANCE OF INEFFECTIVENESS IN THE HOST

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1. INTRODUCTION

THE effectiveness of symbiosis between nodule bacteria and the leguminous plant is usually measured by the growth of the nodulated plant in the absence of combined nitrogen in the root medium. It is thus a measure of the nitrogen fixing capacity of the nodule and ranges from full effectiveness which supports normal plant growth to complete ineffectiveness in which no nitrogen is fixed.

Earlier work has shown that effectiveness depends upon heritable factors in the bacteria and in the host plant. In a previous paper (Nutman, 1954*b*) a recessive host factor (i_1) was described which causes complete ineffectiveness in nodules produced by a strain of bacteria which otherwise gives an effective response in the host. Also, among crosses between effectively responding plants described in the first paper of this series (Nutman, 1954*a*, table 3) some families segregated a larger number of ineffectively responding plants than occurred in unselected material. This paper will concern the further genetic analysis of ineffectiveness in one of these families (fam. 50). The influence of bacterial strain variation on the response of plants of differing genetic constitution will also be considered. Previous papers may be consulted for details of origin of plant and bacterial material and of experimental techniques.

2. OBSERVATIONS, RESULTS AND DISCUSSION

The parents of family 50 were selected in an experiment carried out in 1941 with commercial late-flowering Montgomeryshire red clover inoculated with *Rhizobium trifolii*, Rothamsted strain A, and grown on agar in test tubes under bacteriologically controlled conditions. One parent referred to below as P_3 (to distinguish it from the ineffective plants P_1 and P_2 described previously) responded completely ineffectively with this strain ; the other parent responded effectively. The ineffective parent had 25 nodules of small size (mean length 0·67 mm.) and the effective parent only three nodules of unusually large average size (2·76 mm.).

The progenies of the cross between these plants and of subsequent

crosses were tested in bacteriologically controlled culture in test tubes inoculated in each case with the effective bacterial strain A.

(I) *Genetic analysis of families derived from the ineffective P_3*

The results are given in table 1 using the five size grades of host response previously described. These comprise grade 0, which

TABLE 1

Response of progenies derived from the original ineffective plant P_3 to inoculation with strain A

Family number	Cross type I, ineffective ; E, effective	Response of progeny : no. of plants in each grade					Corrected ratios I : E
		0 (I)	1	2	3	4 (E)	
50	Parental cross : $P_3 \times$ unrelated effective	4	1	3	5	20	4·5 : 28·5
149, 150	Backcross of E (F_1) × E (Parent)	38	7	8	16	34	41·5 : 61·5
151	Backcross of I (F_1) × E (Parent)	12	1	3	6	6	12·5 : 15·5
152	Sib cross : E (F_1) × E (F_1)	21	2	7	7	15	22 : 30
153	Sib cross : I (F_1) × E (F_1)	10	3	1	2	4	12·5 : 8·5
154-166	E × E and E × I Sib crosses in F_2 and F_3	103	15	12	52	60	110·5 : 151·5
167-171	Sib crosses : I (F_3) × I (F_2) (1)	97	4	...	...	...	...
172-181	Sib crosses : I (F_3) × I (F_3) (2)	79	11	7	12	33	84·5 : 57·5
182	I (F_3) self	2	...	...	...	...	...
183	I (F_2) outcrossed to unrelated E	...	...	1	4	24	...
184, 185	I (F_2) outcrossed to unrelated E	...	...	3	8	38	...
186-190	Offspring of fam. 184 *inter se*	30	13	29	27	38	36·5 : 100·5
191-198	Offspring of fam. 185 *inter se*	26	13	22	60	28	32·5 : 116·5
199-201	Backcross of E (fam. 184) × I parent	73	20	13	14	19	83 : 46 (+26 albinos)
202	Backcross of I (fam. 186) × E (fam. 183)	18	1	...	10	3	18·5 : 13·5
203	Backcross of I (fam. 186) × E (fam. 183)	...	...	2	10	7	...

consists of completely nitrogen starved plants indistinguishable from uninoculated controls ; grade 4 which contains plants fully effective in response to inoculation and showing no symptoms of nitrogen deficiency ; grades 1, 2 and 3 show intermediate degrees of effectiveness. For the purpose of estimating segregating ratios in progenies containing intermediately graded plants, the plants classed in grade 1 are regarded as half effective and half ineffective and those in grades 2, 3 and 4 as effective. This classification is based upon a study of the relation of these gradings to the normal distributions of dry weight in large populations of effective and ineffective plants (see Nutman, 1954*a*, p. 41).

Family 50 is shown in the table as a parental cross with later generations conventionally designated.

The response of the parental cross was itself mixed, 14 per cent. falling into the ineffective category. The original P_3 plant was short lived so that backcrosses could not be made with it, and of the four

wholly ineffective F_1 progeny of family 50 only one survived for further crossing, with the result that crosses among ineffectives could not be carried out at this stage. However, effective progeny of the primary cross and the single surviving F_1 ineffective were backcrossed to the effective parent (fams. 149, 150, 151) and these gave rise in each kind of cross to approximately equal numbers of the contrasted types. F_1 segregants in family 50 were also crossed among themselves (fam. 152) and a single family (fam. 153) was raised from unlike parents. In both of these crosses effectives and ineffectives again segregated in equal numbers. Later crosses among F_2 and F_3 effectives and between effectives and ineffectives in F_2 and F_3 were carried out with substantially the same result as in earlier generations, about equal numbers of effectives and ineffectives appearing in the progenies (fams. 154-166).

The ineffective segregants in these crosses generally did not long survive transplanting from agar into soil. It was not until the F_3 generation that more than one surviving ineffective reached the stage of flowering and setting seed. These on intercrossing (fams. 167-181) gave progenies which were either wholly ineffective or about one-third effective. Among the crosses between F_3 effectives and between plants of differing response, some of the families segregated a much higher proportion of effectives than others. The relatively simple ratios seen in many of these crosses suggest that simple genetic factors are involved, their segregation being obscured by other hereditary influences.

Outcrosses were next carried out between F_2 and F_3 ineffectives and unrelated effective plants (fams. 183-185). These were wholly effective and demonstrated the recessive nature of ineffectiveness in the foregoing progenies. Effectives from families 184 and 185 crossed among themselves produced one ineffective in every four examined. The first group of families was very variable giving a $\chi^2_{[4]}$ of 24·3 ($P = 0{\cdot}1$) in spite of a good average agreement with a 1 : 3 ratio; families 191-198 were more homogeneous ($\chi^2_{[7]} = 11{\cdot}8$, n.s.). This suggests that ineffectiveness in these families is unifactorial.

Ineffectives after outcrossing were again difficult to raise to maturity; of seven planted out into soil only four survived and of these three failed to flower. Backcrosses involving effectives from family 184 and an ineffective from family 186 gave variable results due to the segregation of albinos, the symbiotic response of which could not be determined. The number of albinos corresponded approximately to that required to restore the observed ratio to unity as shown in the table. There seems little doubt therefore that the departure from 1 : 1 in these families is due to linkage between the dominant effective factor and an albino factor. Backcrosses were also made between ineffectives from family 186 and effectives from family 183. One of these (fam. 202) gave the expected equal numbers of effectives and ineffectives. The other (fam. 203), however, was

wholly effective ; this may be because the ineffective parent in this case owed its ineffectiveness to factors other than the primary gene which appears to be segregating in these families.

In the previous paper (Nutman, 1954*b*) data were presented to show that the expression of ineffectiveness due to a factor i_1 was subject to modification by an independent suppressor gene m_1 in such a way that the genotype $i_1i_1m_1m_1$ was effective. The failure to account for the segregations found in families 149-166 in terms of a single factor suggests that some kind of genetic modification of the action of a primary gene may also be concerned here.

TABLE 2

The influence of inbreeding on effectiveness

Number of families	Number of plants	Wright's * coeff. of inbreeding per cent.	Grading distribution (per cent.)				
			0	1	2	3	4
Pedigree 1							
...	116	outcrosses	4	...	2	7	87
9	195	25·0	3	4	14	20	59
Pedigree 2							
...	228	outcrosses	1	2	6	10	81
6	159	3·1	3	3	4	17	73
2	66	6·3	...	3	21	18	58
2	36	15·6	5	...	31	17	47
12	266	25·0	3	3	14	24	56
Pedigree 3							
...	63	outcrosses	...	2	10	23	65
2	20	12·5	...	...	5	35	60
5	55	25·0	7	13	18	34	28
1	30	37·5	7	7	10	43	33

* Wright, 1922.

A suppressor gene will tend to give rise to a preponderance of effectives in crosses either among effectives or between ineffectives and effectives. In families of ineffective parentage such a factor will lead to a proportion of families in which about equal numbers of effectives and ineffectives segregate in progenies which otherwise would be pure breeding for ineffectiveness. This explanation is not at variance with the results obtained although a number of wholly effective families would have been expected to occur in the number examined.

Alternative hypotheses would be that more than one simple ineffective gene is concerned or that effectiveness is multifactorial and shows incomplete dominance. In the former case a minority of wholly effective families would be found in the effective × effective and effective × ineffective crosses, the rest segregating about equal

numbers of effectives and ineffectives. On this hypothesis also, the ineffective × ineffective crosses would be either wholly ineffective, or give rise to variable proportions of effectives.

If multifactorial inheritance is concerned, some data on the effect of inbreeding are relevant. These are set out in table 2 and refer to three separate pedigrees (unrelated to any of the above material) in which no clear segregation of ineffectives occurred. The results show that inbreeding leads to a progressive reduction in effectiveness in each pedigree, the proportion of plants falling into the intermediate categories increasing but the number of fully ineffective plants remaining fairly small. Some of the ineffectives appearing in families 149-166

TABLE 3

Relation between the genes i_1 *and* ie

Family number	Cross type	Response of progeny					Corrected ratios
		0	1	2	3	4	
204	$P_1 \times P_3$; I × I . .	...	...	...	...	11	...
205	Derived $i_1 i_1 \times P_3$; I × I .	...	...	...	...	4	...
	Derived $i_1 i_1 \times$ *derived* ie.ie ; I × I						
206-210	Expt. (1) . . .	14	15	21	13	18	21·5 : 59·5
206-210	Expt. (2) . . .	4	...	...	25	107	4 : 132
	F_2 of $i_1 \times$ ie *cross* :						
211-214	E × E . . .	52	3	5	14	21	53·5 : 41·5
215-218	E × I . . .	57	5	7	23	27	59·5 : 59·5
219	I × I . . .	30	1	1	...	...	...

are probably of polygenic origin but the results in table 2 suggest that the number of such ineffectives would be too few to obscure the segregation of major genes.

A detailed reconsideration of segregation in the F_1 to F_4 generations in the light of these hypotheses will not be attempted ; the families are too small to allow critical tests to be made, but the evidence seems sufficient to justify the tentative designation of a recessive gene (*ie*) for ineffectiveness in this material. Information on nodule size within these families is given below and bears upon the identity of the gene *ie* and its possible modification by other host factors.

(ii) *The relation between the factors* i_1 *and* ie

The results of breeding tests made to determine the relation between the factors i_1 and *ie* are given in table 3.

That these factors are neither identical nor allelic is suggested by the absence of ineffectives in two small families (fams. 204, 205) raised from crosses between the original ineffective selections P_1 and P_3, and between a derived i_1 homozygote and P_3. However, later

hybrids (fams. 206-210) between derived i_1 and derived *ie* plants were not completely effective, the proportion of ineffectives appearing in the progenies varying from test to test as shown in table 3. This may indicate either that some change has taken place in the bacterial strain or that other factors for ineffectiveness, susceptible to environmental influences, are segregating in these lines. As no indication of change in the bacterial strain could be inferred from other experiments carried out subsequently (see table 7), selections were made to test the second hypothesis. The results (fams. 211-219) of tests carried out at different times show that crosses among effectives, and between effectives and ineffectives, segregate approximately in the ratio of 7 ineffectives to 9 effectives. The single cross between ineffectives bred true so that there are other hereditary influences contributing to ineffectiveness in this material. Although the data are consistent with a 7 : 9 segregation, they are insufficient to determine whether the genes i_1 and *ie* are independently inherited. Further information on the independent action of these genes is given below in a section dealing with the number and size of nodules found in these families.

(iii) *Characteristics of the* ie *plants*

Homozygotes of type *ie* classified into grade 0 were indistinguishable in top size and in the colour and appearance of their leaves from normal plants inoculated with ineffective strains of bacteria, or from plants of the i_1 line inoculated with strain A, or from uninoculated control plants. At harvest (100 days) the cotyledons and older leaves had withered and the youngest or two youngest leaves were very small and pale yellow green. The seedling root systems of *ie* plants were normal in appearance and nodules formed readily upon them.

(iv) *The number and size of nodules on* ie *plants*

With respect to nodule size and abundance *ie* ineffectives differed from plants inoculated with ineffective strains and from i_1 plants. In general *ie* nodules were variable in number and were not more numerous than nodules on heterozygotes, nor were they exclusively of small size. Two *ie* ineffectives are figured in plate 1, *a* and *b*; they illustrate the range found both in number and size and may be compared with a normal effective (*d*).

The mean number of nodules occurring on *ie* plants showing either ineffective, intermediate or effective responses are set out in table 4 with i_1 material included for comparison.

Among plants of families bred directly from P_3 without outcrossing the mean nodule number shows a maximum for plants of intermediate response. This trend becomes more marked among segregating families after outcrossing; plants of intermediately effective response forming twice as many nodules as completely ineffective plants. The general increase in numbers after outcrossing may reflect the use of rather abundantly nodulating plants as effective parents.

These families thus show a consistent tendency in their nodulation with strain A for both the least effective and the most effective plants to produce the fewest nodules, and the intermediate grades the most. This pattern appears to be specific for strain A since with other strains of varying degrees of effectiveness the homozygous line gives the normal ineffective maximum and effective minimum as shown in table 4. This is brought about by a marked reduction in the number of nodules produced on plants of increasingly effective response and not by an increase in numbers of nodules on ineffectives.

These relations are in striking contrast to those shown in table 4 for i_1 plants upon which very large numbers of nodules were formed with strain A (group 4) and fewer nodules with strains giving an effective response (group 5).

TABLE 4

Average numbers of nodules on plants of ineffective, effective and intermediate response in ie *and* i_1 *lines*

	0	1	2	3	4
(1) *ie* lines (fams. 154-178) before outcrossing, inoculated A strains	64	72	95	83	70
(2) *ie* lines after outcross (fams. 191-198) inoculated A strains	97	140	181	138	119
(3) *ie* lines (fams. 161-171) inoculated with effective and ineffective strains unrelated to strain A	65	78	26	59	36
(4) homozygous i_1 lines inoculated strain A	113	...	...	...	...
(5) homozygous i_1 lines inoculated with effective and ineffective strains unrelated to strain A	130	...	111	64	48

On nodule size also these two factors have divergent effects, as can be seen in fig. 1 which gives distributions of nodule size in *ie* and i_1 lines inoculated with strain A and an effective strain ClF. The nodules on *ie* plants range in size up to 4 mm., whereas with few exceptions the more numerous nodules on i_1 plants are below 1 mm. in length. The *ie* plants in fig. 1 were taken at random from two sets of families (fams. 154, 157 and 172-174). They show that great variation in nodule size occurs even among ineffectively responding plants, and also indicate an interesting relation which was obscured in the results for nodule number only: the *ie* effective segregants with A are aberrant in showing considerable numbers of small nodules *in addition* to an appreciable number of nodules over 2 mm. in length. In fact, in size and in number, the distribution of nodules on these plants is equivalent to the summation of a normal effective nodulation and an ineffective nodulation characteristic of *ie* ineffectives.

The genotypes of the effective plants with strain A are not known in these families. It was suggested above that the response of the recessive homozygote may be modified by a suppressor gene or genes,

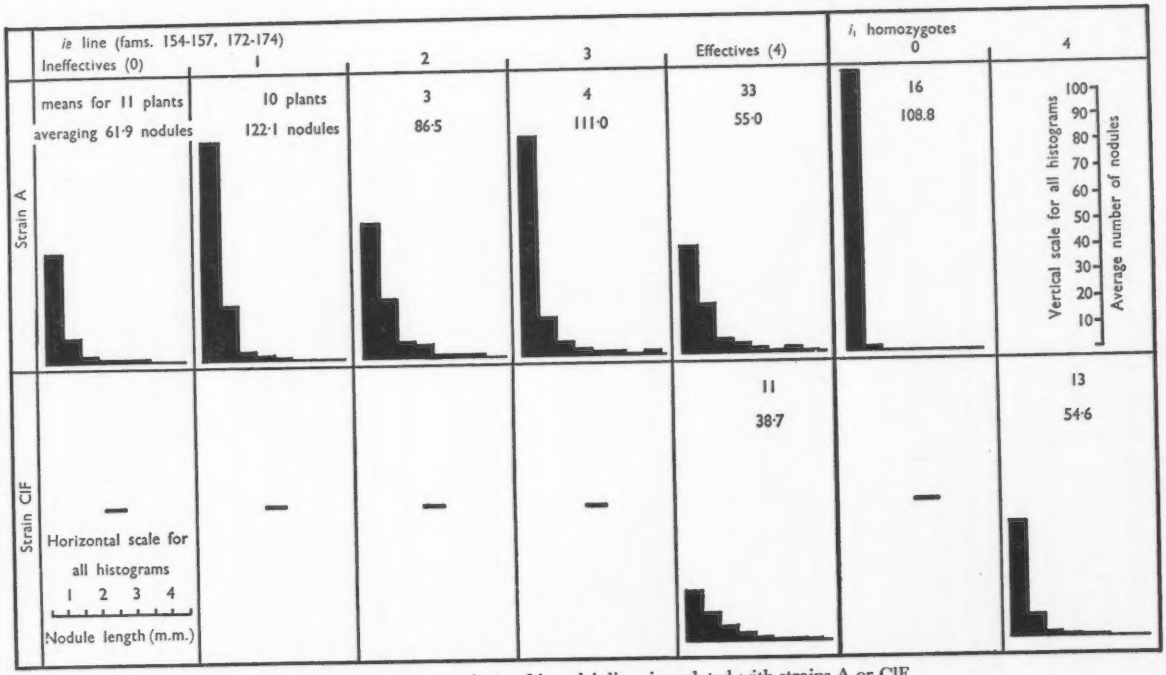

FIG. 1.—Nodule size on plants of *ie* and i_1 lines inoculated with strains A or ClF.

or that these effectives are themselves heterozygous for *ie* or other factors, the genetical evidence being inconclusive. The distribution of nodule size on these effectives would be more readily understood in terms of a modification of the expression of the *ie* gene. Should effectiveness in these families be due in this way to a modifying factor or complex rather than to heterozygosis at the *ie* locus, it would appear that these genes are showing independent effects, the *ie* homozygote giving rise to its particular distribution of nodules upon which is superimposed a normal effective type of nodulation due to the action of other genetic factors.

This interpretation requires that effective and ineffective nodulation by a single strain of bacteria may co-exist on the same root without apparent interference, on the basis that the amount of nitrogen fixed in the fewer effective nodules suffices to sustain normal growth and to allow at the same time the formation of ineffective nodules. The effective nodules would allow development of more foci of infection which would then give rise to a majority of ineffective nodules, the inhibitory influence of the fewer effective nodules being counteracted by their stimulating effect in promoting renewed growth.

In this way these plants would resemble normal plants inoculated with mixed effective and ineffective inocula which will give rise under certain conditions to both many small and few large nodules, as Nicol and Thornton (1942) have shown. This interpretation also suggests that the action of the modifying factors is to induce the effective response only in some nodules, not in all, possibly on certain parts of the root or determined by some physiological balance in the host. It was in fact observed that the development of the effective response was retarded in certain individual plants in which development of the effective response was strikingly correlated with the late formation of typically effective nodules on the root, as shown by the plant illustrated in plate 1, fig. *c*. The appearance of such plants suggests that a complete change in the activity of the new nodules occurred at about the time of formation of the fourth leaf. Plants of this kind occurred infrequently and sporadically. In the absence of any evidence (see below) that a change had occurred in the bacteria, the physiology of the host must in some way be involved. An investigation of the anatomy and cytology of these nodules is being undertaken.

A detailed analysis of nodule size and number is required to elucidate fully these relations, both from the genetical and physiological points of view; the determination of nodule size and number as well as effectiveness is more complex in these families than in the i_1 pedigree.

(v) *The independent determination of the* i_1 *and* ie *types of nodulation*

Measurements of nodule length were made on effective and ineffective plants in F_2 families derived from a cross between i_1 and *ie*

homozygotes ; the ineffective segregants for measurement were selected at random. The results are presented in histograms in fig. 2.

The effective plants showed a wide range of nodule size, occasional nodules measuring more than 5 mm. in length. Variation in size was also considerable among some ineffectives but was much less on others, and on this basis a division of the ineffectives into two groups could readily be made as has been done in fig. 2 ; the arbitrary separation is made at 2 mm. The first group contains plants with a type of nodulation typical of i_1 homozygotes and the second group

TABLE 5

Response of ie *homozygotes (fams. 167-169) and unselected red clover with different strains of nodule bacteria*

Strain	*ie* line response					Mean dry weight (mgm.)	Unselected red clover response					Mean dry weight (mgm.)
	0	1	2	3	4		0	1	2	3	4	
Experiment 1 (*Rothamsted*)												
*f*12	14	1	...	.	...	16·5±1·2	...	14	...	...	...	20·7±0·9
HKC	9	3	...	...	...	17·2±1·5	...	6	5	...	...	21·7±0·7
42	12	3	...	...	...	17·9±0·6	...	1	4	4	1	30·5±3·4
Haldon 1	12	...	1	3	...	19·4±1·3	...	...	2	5	6	44·8±4·7
Hannay 7	3	1	3	1	6	30·9±4·1	...	...	...	2	10	50·5±1·8
ClF	1	1	...	4	6	35·0±4·4	...	...	...	2	11	48·5±2·7
Strain A	8	...	...	1	3	25·2±4·4	...	...	...	1	4	49·0±8·5
Experiment 2 (*Canberra*)												
HKC	7	...	...	...	...	3·6±0·1	7	...	...	...	...	4·4±0·3
SU. 297/3	19	1	1	...	...	9·4±2·9	3	5	10	3	1	33·7±4·3
Haldon 1	22	...	...	...	...	3·8±0·2	...	...	3	11	8	64·5±3·8
ClF	6	1	2	8	10	49·9±6·6	...	...	...	6	15	73·2±1·5
Strain A	20	2	1	2	2	10·9±3·9	...	...	...	1	13	63·4±2·3

those typical of *ie* homozygotes, suggesting that the plants in the first group represent the i_1 segregants and those in the second group the *ie* segregants.

The contrasted types of nodulation found in these ineffectives, in about equal numbers, gives additional support for the unifactorial nature of these genes. Other factors for ineffectiveness may be segregating in these families as suggested above, although there is no evidence from nodule measurement of other distinctive size distributions than those which can be attributed to i_1 or *ie* homozygotes, neither can the i_1, *ie* double recessive be identified.

(vi) *Response of* ie *homozygotes with other strains of bacteria*

The reactions of *ie* plants to inoculation with a number of bacterial strains were examined in two experiments. The results are given in table 5 in terms of the number of plants falling into each category of response, as well as in mean plant dry weight for each combination

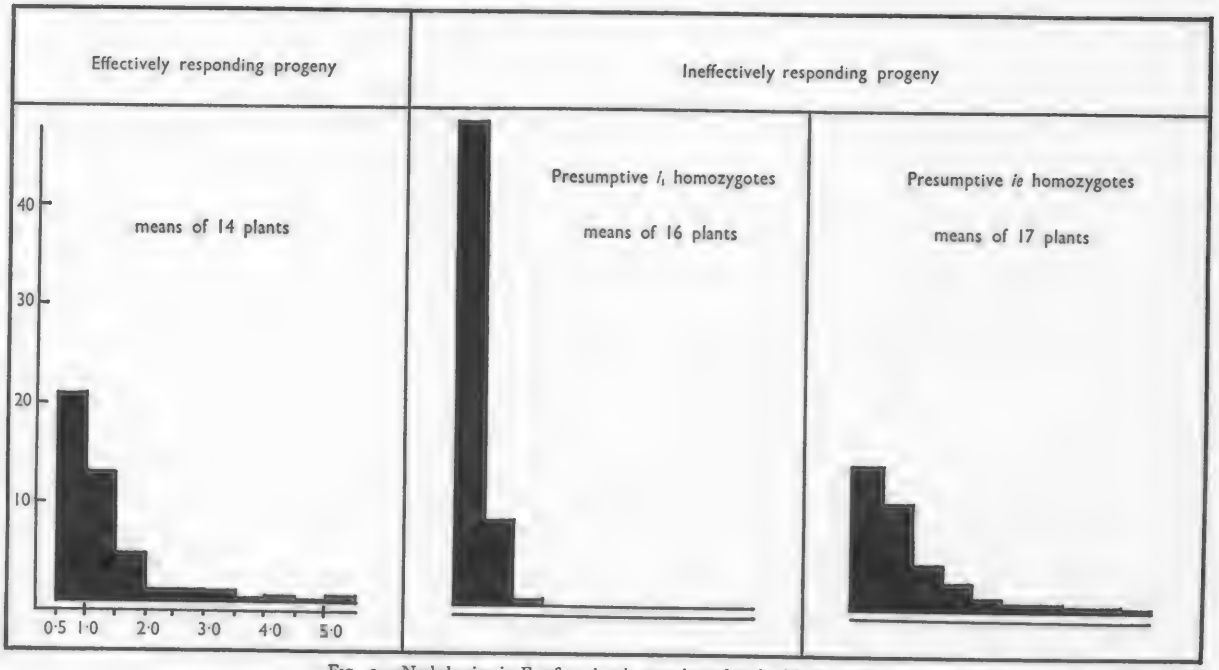

FIG. 2.—Nodule size in F_2 of an $i_1 \times ie$ cross inoculated with strain A.

of strain and host; uninoculated clover is included as control for bacterial strain. The bacterial strains employed were strains A, ClF and Hannay 7 as effectives, strains Haldon 1, SU.297/3 (Baird, 1955) and Rothamsted 42 as intermediates, and Rothamsted strains HKC and *f*12 as ineffectives (the strain *f*12 is a mutant ineffective derived from strain A and has been found to give a generally similar response to the standard ineffective strain HKC—Nutman, 1946). The results of the experiments are in agreement and show that the *ie* line responds in an almost completely ineffective manner with all strains of less effectiveness than A. On the other hand, with the two most effective strains the *ie* homozygotes are mostly effective, although they do not reach the degree of effectiveness found on unselected material.

TABLE 6

Response of families in the ie *line which derive from a plant responding ineffectively with strain ClF*

Family	Cross type	Response of progeny											
		with strain A						with strain ClF					
		0	1	2	3	4	Corrected ratio	0	1	2	3	4	Corrected ratio
220	I (with A) × I (with ClF)	13	3	5	5	4	14·5 : 15·5	5	1	4	16	3	5·5 : 23·5
221	„ „	24	...	1	...	...	...	14	2	2	4	...	15 : 7

It is of interest that with Hannay 7 and ClF the *ie* homozygotes do not respond uniformly; some continue to show a fully ineffective response whereas others are quite effective. This suggests that the suppression of the interaction between the *ie* gene and factors in the bacteria leading to ineffectiveness, is not only a function of bacterial strain but also involves other host determinants, which in this instance may be simple and dominant (χ^2 (1 : 3) = 1·01, n.s.). To examine the possibility of further selection towards ineffectiveness in the *ie* line, a plant responding ineffectively with ClF was crossed with two normal ineffectives (inoculated strain A). These families were tested against strains A and ClF.

The results of this test given in table 6 show that the two families behaved differently; one continued to show about one quarter ineffectives with ClF (χ^2 = 0·56 n.s.), and also segregated 1 : 1 with strain A. The other responded in a largely ineffective manner with ClF. This result clearly indicates that simple genetic factors in the host determine whether the interaction between the *ie* gene and the bacteria will lead to ineffectiveness or effectiveness. It is also evident from the parallel family differences with strain A that these factors are non-specific.

This absence of a high degree of specificity with respect to strain is in marked contrast to the results found for i_1 where the specificity with respect to strain A was complete.

(vii) *Response of* ie *homozygotes with strain A re-isolates*

In tests made with *ie* line plants no opportunities have arisen for the isolation of mutant strains of bacteria from single large effective nodules on plants otherwise bearing only large numbers of small nodules, as occurred with the i_1 line. As noted above *ie* plants tended to produce numbers of large nodules as well as ones of medium or small size. Isolations were made from the largest and smallest nodules available. Included with these were isolates from large nodules on plants bearing larger nodules on the younger parts of the root instead of on the crown, a type of nodulation which occurs infrequently on the *ie* line and is illustrated in plate I, fig. *c*. Strain A was also passaged repeatedly through the *ie* host and then tested for effectiveness. Isolates were taken at each passage from a number of nodules to increase opportunities for adaptation.

The response of these various classes of re-isolate was examined on *ie* plants (including families segregating small numbers of effectives) with the results set out in table 7. At the same time tests were made with re-isolates from nodules on i_1 plants, two of which (substrains A211 and A421643) had previously been shown to respond more effectively with i_1 plants.

No consistent differences appeared in the effectiveness of any of these re-isolates with *ie* plants. The strain A211 appeared in the first test to be a little more effective than A, but this was not confirmed in the later experiments. In view of the high degree of specificity of the effective response of A211 with i_1 and its dependence also upon factors other than i_1 (see Nutman, 1954*b*, p. 54), the result obtained was not surprising.

The tests of the new re-isolates from *ie* nodules were also negative as far as the detection of any alteration in effectiveness was concerned, whether they were derived from effectively or ineffectively responding plants, or from large or small nodules, or had been carried through a number of plant passages.

The plants grown at Canberra, under conditions of higher temperature and light intensity, gave a more marked ineffective response than those in the Rothamsted experiments. This also appears in the dry weights recorded in the two experiments in table 5, where, however, the comparison between bacterial strains is not affected. The low final dry weights of the ineffective plants in the warmer environment may be due to higher respiratory losses.

3. GENERAL DISCUSSION

(i) *Genetics of symbiotic effectiveness*

Earlier results have shown that effectiveness of the host's response

in symbiosis may be determined by primary genes and by associated modifying factors, each of which interact specifically with factors in the bacterial strain.

TABLE 7

Response of ie *homozygotes with re-isolates of strain A taken from nodules on* i_1 *or* ie *homozygotes*

No. of isolates tested	Origin and characteristics of isolate where previously tested	Response of *ie* plants (fams. 167, 168)				
		0	1	2	3	4
	(1) *Isolates from* i_1 *nodules* (see previous paper, tables 3 and 6)					
...	Rothamsted strain A control	19	1	...	...	...
1	Mutant strain effective with i_1 host (substrain A211)					
	Expt. 1	5	2	1	1	3
	Expt. 2, 3	53	2	1	1	...
1	Ineffective with i_1 (substrain A421)	12	1	...	1	...
1	Partially effective with i_1 (substrain A421643)	12	1	...	...	...
	(2) *Isolates from* ie *nodules*					
	(*a*) *Isolates from plants with larger nodules on older roots*					
1	from medium size nodule on effective host	16	2	...	...	...
4	from large nodules on ineffective plants	33	4	2	2	1
2	from small nodules on ineffective plants	22	2	1	...	...
	(*b*) *Isolates from plants with larger nodules on younger roots*					
1	from large nodule on intermediately effective plant	20	3	1	...	1
1	from small nodule on same plant	19	...	...	...	...
3	from large nodules on ineffective plants	35	4	1	...	1
1	from small nodules on ineffective plants	13	3	...	...	...
	(*c*) *Serial plant passage re-isolates*			*family* 192		
...	Original stock culture without plant passage	11	1	...	3	4
...	from *ie* nodules after 1 serial plant passage	2	...	1	...	...
...	from *ie* nodules after 2 serial plant passages	3	...	...	...	1
...	from *ie* nodules after 3 serial plant passages	3	...	...	...	...
...	from *ie* nodules after 4 serial plant passages	4	1	1	...	...
...	from *ie* nodules after 5 serial plant passages	5	...	1	2	...
...	from *ie* nodules after 6 serial plant passages	2	...	2	1	2
...	from *ie* nodules after 7 serial plant passages	6	2	1	1	...
...	from *ie* nodules after 8 serial plant passages	8	...	1	...	...
...	from *ie* nodules after 9 serial plant passages	3	...	3	...	...
				family 167		
...	Original stock culture without plant passage	39	1	...	...	...
...	from *ie* nodules after 6 plant passages	27	...	...	...	...
...	from *ie* nodules after 9 plant passages	24	2	...	...	...

This interpretation receives support in the present paper by the identification of a second primary gene for ineffectiveness, *ie*, and by the suggestion that its action may also be influenced by modifiers. The gene *ie* is, however, largely unspecific in its interaction with bacterial strain, as also is the action of modifying genes.

The independent action of these genes is inferred from a consideration of the divergent types of nodule ascribed to each of them (fig. 2), and by their differing specificities with regard to bacterial strain.

Although the ineffectiveness induced by i_1 or *ie* appears to be due to effects on the tissue of the nodule containing the bacteria, and in which nitrogen is presumed to be fixed, the extent to which these effects involve the uninfected growing point of the nodule differs decisively. With i_1 the nodule meristem is immediately involved so that the nodule makes no growth and remains very small. With *ie*, on the other hand, the processes taking place in the infected tissues do not involve the nodule meristem to the same extent so that growth continues and larger nodular structures are produced.

The relationship between nodule size and abundance conforms broadly to previous experience : the larger the average nodule size the smaller the number of infections sustained. An explanation of this relationship in terms of the morphogenesis of nodules from incipient lateral root initials and their inhibition by the activity of nodule and root growing points has been presented elsewhere (Nutman, 1948, 1952). The correlation between abundant nodulation and small nodule size in much of this material gives additional support for this theory.

The modes of action of the modifiers presumed to influence the genes i_1 and *ie* also show interesting differences. The modification of the action of *ie* with strain A appears to operate by inducing the effective response in a proportion only of *ie* nodules, the remainder continuing to show ineffective morphology and function. As a result, ineffective nodulation is able to continue on a root system which is expanding on account of the presence of effective nodules. Consequently a much larger number of nodules is produced than can be formed on a wholly ineffective plant. An investigation of the physiology of this situation may reveal the presence of mobile substances which stimulate or inhibit effectiveness.

In contrast the factor m_1 has been shown to nullify completely the effect of the i_1 gene so that relatively few nodules of average size are formed and typically ineffective nodules are absent (table 7 of the previous paper, fams. 138, 139). In this connection it may also be noted that the nodules on effective *ie* plants inoculated with strain ClF were typically effective in distribution and size (table 4 and fig. 1). If effectiveness in these plants is determined by supplementary dominant factors, as seems probable from the data in table 6, such factors act like m_1 upon the plant as a whole. Further work may show that modifying factors are of greater significance in the development of symbiosis than the primary genes, although the latter individually appear to show the most striking effects because without them the modifying influences cannot be detected.

(ii) *Viability of ineffective selections*

The development of ineffectiveness in the *ie* material was not correlated with any obvious characteristics in the young seedling plants before nodulation commenced. Moreover, homozygotes

inoculated with the very effective strains ClF or Hannay 7 continued to develop in a normal manner. Nevertheless considerable difficulty was met in raising the homozygous *ie* plants, inoculated with strain A, to maturity. Only nine ineffectives survived out of a total of more than 60 planted into soil and of these some failed to flower. This could not be attributed to their initial small size and N-starved condition since other ineffective selections and plants inoculated with ineffective strains were readily grown into mature plants.

In the i_1 line any weakening effect of the homozygote was not at first noticed. Of the nine ineffectives of the original family in the i_1 pedigree (Nutman, 1954*b*, table 1, fam. 81) which were planted into soil, only one was lost. Similarly in families derived directly from family 81 no considerable losses occurred. After outcrossing, however, the semi-lethal effect of the homozygote was very evident and of fourteen selected plants only three survived.

Why are these recessives so inviable? The results recall those obtained for the differential survival of plants resistant to infection by nodule bacteria (*r* homozygotes) in the presence and in the absence of a specific cytoplasmic factor (σ cytoplasm) previously described (Nutman, 1949). It was there shown that *r* was lethal except in the presence of σ cytoplasm. This cytoplasm was also shown to have a similar effect on the viability of a number of abnormal seedlings.

It is probable that the difference in survival of the i_1 homozygotes before and after outcrossing was due to the same cytoplasmic factor because one of the original parents (P_1) was a daughter of a resistant plant, and was shown to contain σ cytoplasm by independent breeding tests. On outcrossing this cytoplasm was lost and the viability of the ineffectives decreased. Although the poorer longevity of *ie* plants compared to i_1 ineffectives before outcrossing may be similarly due to disturbances in metabolism of cytoplasmic origin, there was no evidence in the *ie* pedigree of maternal effects which might be associated with differences in cytoplasmic components.

4. SUMMARY

1. A recessive gene (*ie*) is described which confers symbiotic ineffectiveness upon plants inoculated with certain effective strains of nodule bacteria.

2. The ineffective response in *ie* hosts is not markedly specific to bacterial strain; with outstandingly effective strains a mixed response occurs, with others the response is uniformly ineffective.

3. No variants of the test strain A have been found with which *ie* homozygotes are effective.

4. *ie* nodules produced by strain A vary in size and abundance from plant to plant.

5. Modification of the *ie* response with strain A by other host factors is tentatively inferred from the genetical results. This modification does not appear to suppress fully *ie* type nodulation with strain A.

Plate

FIGS. *a* and *b*.—Ineffectively responding *ie* plants inoculated with strain A.

FIG. *c*.—Modification of the ineffective response in *ie* homozygotes with strain A by other host factors acting on younger nodules. Change in response in plant "c" occurs at about 4th leaf stage. Note correspondence between changes in top growth and nodule pattern.

FIG. *d*.—Effectively responding *ie* heterozygote.

Fig. *a*

Fig. *b*

Fig. *c*

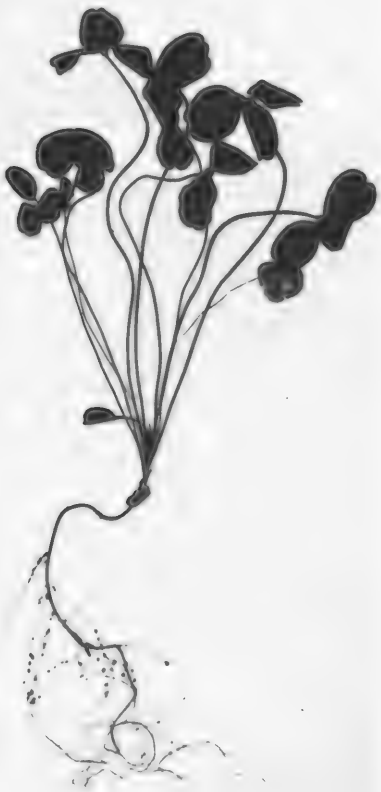

Fig. *d*

6. Ineffectiveness due to *ie* differs from that due to the factor i_1 previously described in (*a*) the size and numbers of nodules formed, (*b*) specificity with respect to bacterial strain, and (*c*) the mechanism of its suppression by other host factors. The genes i_1 and *ie* are neither identical nor allelic.

7. The factor *ie* decreases the vitality and longevity of the host and affects flowering. Possible reasons for this are discussed.

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SYMBIOTIC EFFECTIVENESS IN NODULATED RED CLOVER

IV. THE INFLUENCE OF THE HOST FACTORS i_1 AND *ie* UPON NODULE STRUCTURE AND CYTOLOGY

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1. INTRODUCTION

In previous papers Nutman (1954*b*, 1957) has described two independent recessive host genes (i_1 and *ie*) each of which causes ineffective symbiosis

TABLE 1

Symbiosis with bacterial strain

Plant lines	Bacteria strains					
	Related strains			Unrelated strains		
	A	A211	*f*12	ClF	SU297	Haldon 1
Controls : unselected or heterozygous plants	E	E	I	E	EI	EI
i_1 i_1 (fam. 148) * .	I	E	I	E	EI	EI
ie ie (fam. 167) † .	I	I	I	E and I	I	I

E = effective response
I = ineffective response
EI = intermediate response
* Nutman, 1954. † Nutman, 1957.

between red clover plants and the normally effective strain of *Rhizobium trifolii*, Rothamsted strain A. These symbioses are characterised by failure in nitrogen fixation. This paper gives the result of cytological investigations of the structure of nodules on plants homozygous for each of these genes. Comparison is also made between these nodules and normal ones produced by other bacterial strains upon the same plant material, and by the effective strain A upon unselected clover.

Plants were grown in test tubes as described previously (Nutman, 1949) using several families of red clover homozygous for i_1 and for *ie* as well as heterozygotes and unselected families as controls. Plants were inoculated soon after germination with one or other of a number of known strains of nodule bacteria giving the responses shown in the table. As the plants developed the position and date of appearance

of each nodule was noted on drawings of the root systems. Plants were taken at weekly intervals and nodules of known age were fixed in Flemming's solution and embedded in paraffin wax. Serial microtome sections (5-7 μ) were cut and stained with Heidenhain's hæmatoxylin or with rose bengale and light green (Brenchley and Thornton, 1925).

2. STRUCTURE AND CYTOLOGY OF CONTROL EFFECTIVE NODULES

The structure and cytology of control effective nodules studied in this investigation agree in every respect with earlier published descriptions; these may be summarised as follows: The effective clover nodule (plate I fig. 1) is an elongate cylindrical outgrowth of the root consisting of an uninfected cortex of diploid cells enclosing an inner tetraploid tissue which contains the bacteria, (Wipf and Cooper, 1938). An endodermal sheath surrounds the infected tissue and separate endodermi enclose each vascular strand in the cortex. The nodule grows at its tip which is meristematic and the progressive infection of new cells produced by the meristem takes place distally from infection threads extending from the already infected cells. The threads consist of minute tubes of cellulose laid down by the plant; these contain the bacteria embedded in slime. The bacteria are discharged into the host cells (usually near the nucleus) from vesicles which arise upon the thread. After their release, the bacteria (rod form) multiply in the host cytoplasm and then enlarge until they completely occupy the cytoplasm of the enlarged host cell or leave only a small vacuole. Bacteria in this form constitute the so called "bacteroids" presumed to be the active agents in fixation (plate I, fig. 4). Bacteroids isolated from the host cell are unable to grow on artificial media (Müller and Stapp, 1925; Almon, 1933), although they react with vital stains (Spicher, 1954). The host nucleus of the infected cell first enlarges and then becomes amœboid, the nucleolus losing its

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Plate I

FIG. 1.—A section of an effective nodule produced by strain CIF bacteria on an *i*, plant. *m* = meristem; *v* = vascular strand; *b* = bacteroid containing tissue. ×300.

FIG. 2.—A section of an ineffective nodule produced by strain *f*12 on a heterozygous plant. *m* = meristem; *v* = vascular strand; *tb* = transient bacteroid containing tissue; *db* = degenerating bacterial tissue. ×300.

FIG. 3.—Bacteroid containing cells in a section of an effective nodule produced by strain A bacteria on a heterozygous plant. ×1600.

FIG. 4.—Bacteroid containing cells in a section of an effective nodule produced by strain CIF bacteria on an *ie* plant. ×1600.

FIG. 5.—A section of an ineffective nodule produced by strain A bacteria on an i_1 plant. *m* = meristem; *v* = vascular strand; *b* = cells containing rod-shaped bacteria. ×300.

FIG. 6.—A high-power photograph of an i_1/strain A nodule showing rod-shaped bacteria in the cytoplasm. *i* = infection thread; *b* = bacteria in the cytoplasm. ×1600.

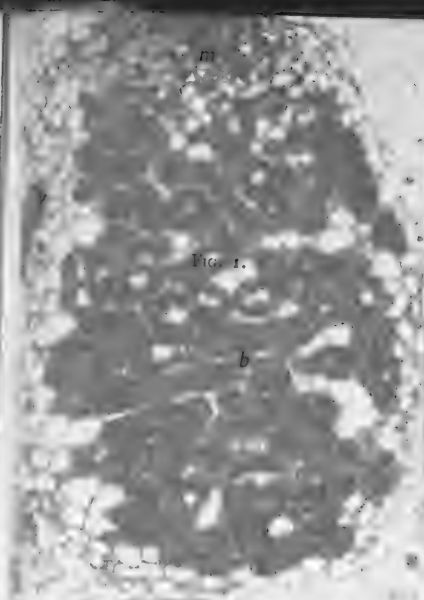


Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

identity and being replaced by a number of particles of chromatinic material. Parallel with the hypertrophy of the host cell nucleus, changes occur in the nuclei of the bacteroids (Bergersen, 1955). The infected cells of the nodule contain hæmoglobin, the function of which is uncertain ; this imparts a pink colour to the nodule. Starch does not accumulate to a marked degree in effective nodules.

As the nodule becomes older, bacteria from the infection threads remaining at the base of the nodule invade the intercellular spaces and with the breakdown of the bacteroids ultimate degeneration of the tissue occurs.

The commonest kind of ineffectiveness in the nodule has been ascribed to bacterial strain ; for example in nodules formed by strains of bacteria from another species or sub-group of the host (Vincent, 1954), or in nodules formed by originally compatible bacteria which have lost the ability to fix nitrogen (Nutman, 1946). Such nodules are usually characterised by earlier decay of the bacteroid containing tissue as with the ineffective variant of strain A (strain *f*12) on control plant material (plate I, fig. 2) although other types of ineffectiveness are known (Bergersen, 1955, and unpublished observations). Thus in general the amount of nitrogen fixed is directly related to the amount of bacteroid containing tissue found within the nodule (Chen and Thornton, 1940).

3. THE NODULES OF i_1 PLANTS

The structure and cytology of ineffective nodules produced on i_1 plants by bacterial strain A differed strikingly from the above description, although infection and the initial differentiation of the tissues of the nodule proceeded in a normal manner. The infection of the individual host cell from the infection thread was also normal as was the multiplication of the rods within the cytoplasm. However, instead of giving rise to bacteroids following initial multiplication, the bacteria remained in the rod form (plate I, figs. 5 and 6). Degeneration of the infected tissue occurred earlier than in normal effective nodules (20-25 days) but later than in nodules produced by typically ineffective strains such as *f*12 (7-10 days). The growth of the i_1 strain A nodule ceased at an early stage and as a result further infection of the root continued at a high rate (Nutman, 1954). The reason for the cessation of growth is not known but does not appear to be due to the ultimate invasion of the meristem by infection threads as occurs with *f*12 nodules. Changes in the host nucleus prior to cell degeneration were the same as in effective nodules, *viz.* enlargement followed by change in shape and fragmentation of nucleoli. Starch granules were abundant throughout older nodules.

The specificity of the action of the gene i_1 was manifest also at a cytological level. With either strains ClF or A211, a variant of A with which i_1 homozygotes responded effectively, bacteroid formation

was normal, the actual shape of the bacteroids being characteristic for these strains (plate I, figs. 3 and 4).

With strain *f*12 (a mutant of strain A, ineffective with all plant material, Nutman, 1946) early decay of bacterial tissue was characteristic of the strain, but no bacteroid formation occurred.

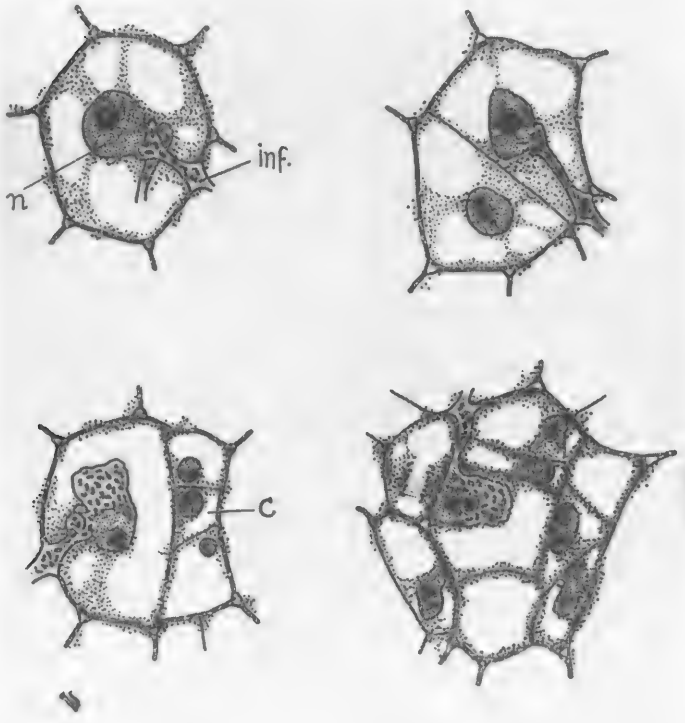
In none of these ineffective associations was hæmoglobin found in amounts which could be detected spectroscopically. From these observations it was concluded that failure to fix nitrogen in i_1 plants inoculated with strain A bacteria was due solely to a host-bacterial interaction which prevents bacteroid formation, the small size of the nodule being secondary.

4. THE INEFFECTIVE NODULES OF *ie* PLANTS

On plants of *ie*/*ie* genetical constitution inoculated with strain A, nodule structure external to the infected tissue was normal and nodules were initiated in the normal way. The central infected tissues, however, consisted largely of small irregularly shaped cells and within these neither the normal invasion and multiplication of the rod form of the bacteria nor bacteroid formation was found. Examination showed that the abnormal cell pattern within the nodule was a result of renewed cell division following immediately upon the release of the bacteria from the infection threads (plate II, figs. 7-11). In a minority of infected cells which did not divide, bacterial multiplication ceased and no further changes occurred in the host nucleus. Where divisions occurred both infected cells and nearby uninfected cells were often involved. In most cases infected cells divided whilst the bacteria were still localised within the cell so that the first division separated the infected portion of the cell from the rest. The uninfected cell thus formed continued to divide a number of times whereas no further divisions generally occurred in the infected cell within which the bacteria seemed to parasitise the nucleus. When, however, first division failed to localise the bacterial infection, further division of the infected cell occurred to give rise to a group of small infected cells. Except in special instances, to be noted below, the bacteria in these cells remained in the rod form, and did not multiply to any great extent. The infected host cells showed a reduced affinity for the light green stain (plate II, fig. 7). These reactions of the host to the release of the bacteria from the infection thread were initiated near the nodule meristem, but the meristem itself was quite unaffected by them and continued its development in spite of an increasing degree of nitrogen starvation in the host. In contrast therefore to most ineffective nodules, *ie* nodules were large structures. Presumably because of the continued activity of the meristem, further infection of the root was inhibited and nodules were relatively sparse (Nutman, 1956).

As a result of the above sequence of events within the nodule the proportion of infected cells was less than in an effective nodule of

comparable size, but the total number of cells was larger. With the exception of " parasitised " cells no degeneration of the host nuclei occurred, and even in these cells normal resting nuclei were sometimes found. No hæmoglobin could be detected in these nodules.



The apparent tumorisation of regions of the nodule about centres of infection also occurred where bacteria multiplied to any extent in the intercellular spaces. An example of this reaction in the host

TEXT-FIG. 1.—A diagrammatic representation of the division of cells in an *ie* ineffective nodule.

1. The initially infected cell.
2. An infected cell which has undergone division after the release of bacteria from the infection thread.
3. Further division of the uninfected cell resulting from the first division : the bacteria are parasitizing the nucleus of the infected cell.
4. The final stage in which the infected cell is surrounded by dividing cells.

n = nucleus ; *c* = uninfected dividing cells ; *inf.* = infection thread.

is shown in Plate II, fig. 11. The intercellular mass of bacteria was here very considerable and cells immediately adjoining the site of intercellular invasion were observed to be unaffected. The stimulus to division occurred at some distance from the infection and has given rise to a complete cambial layer isolating the invasion. This type of reaction occurred most frequently in nodules produced by the strain A211.

Because of the great range in size of nuclei found within the nodule it was at first thought that the secondary cell divisions were associated with a breakdown of the tetraploid condition. Observation showed that mitoses were normal, no abnormal anaphase or telophase patterns were seen and chromosome counts of metaphase plates confirmed the tetraploid condition of these cells (Plate II, figs. 9 and 10).

The ineffectiveness in *ie* homozygotes was previously shown not to be confined to strain A, but was found also with a number of other effective and intermediate strains shown in table 1. In every case the nodule pattern of ineffectiveness in *ie* plants with these strains was found to be similar to that described for strain A.

The action of the *ie* gene may thus be visualised as predisposing the host cells to division, the actual stimulus to division being the release of the bacteria from the infection threads into either the host cell itself or the intercellular spaces. In both situations the bacteria were in contact with the host protoplast either directly or through plasmodesmata. No reaction occurred in cells penetrated by unbroken threads.

5. EFFECTIVE AND PARTIALLY EFFECTIVE NODULES ON *ie* PLANTS

An effective response was found with strain A on occasional plants of the *ie* line, with the formation of more nodules than occur on ineffectives. Such associations were either effective or partially effective from the outset or showed a change in response from ineffectiveness in the younger seedlings to full effectiveness in older plants. In some seedlings the change in response in the top growth of the plant corresponded to a marked change in the pattern of

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Plate II

FIG. 7.—A section of an *ie*/strain A nodule showing regions of tumorisation. ×330.

FIG. 8.—A cell wall tracing of the section shown in fig. 7. The dark-shaded areas represent groups of released bacteria.

FIG. 9.—A high-power photograph of part of a section of an ineffective, *ie*/strain A nodule, showing an infection thread crossing a cell whose nucleus is in metaphase. ×1700.

FIG. 10.—As in fig. 9 but the metaphase plate is perpendicular to the plane of the section. The bacteria released from the infection thread are clearly seen. ×1700.

FIG. 11.—A section of an ineffective nodule produced by strain A211 bacteria on an *ie* plant. *m* = meristem ; *i* = intercellular space filled with bacteria and surrounded by uninfected dividing cells. ×330.

Fig. 7.

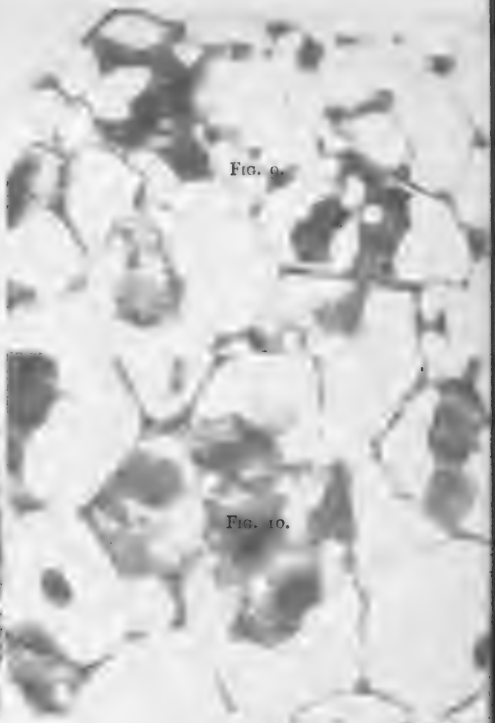


Fig. 9.

Fig. 10.

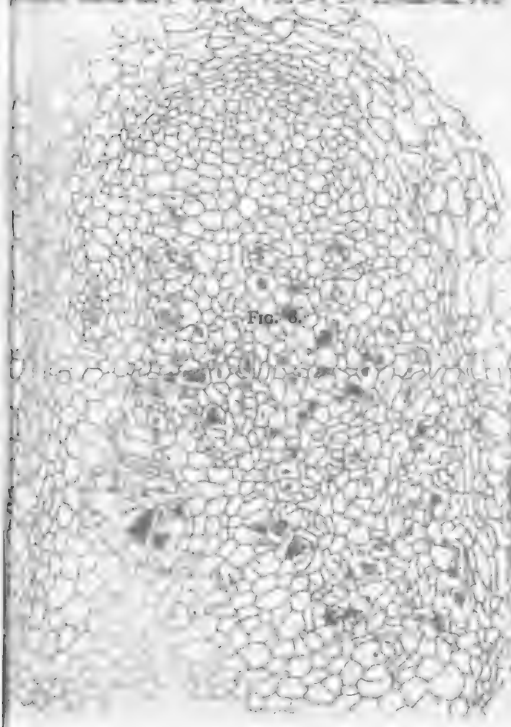


Fig. 8.

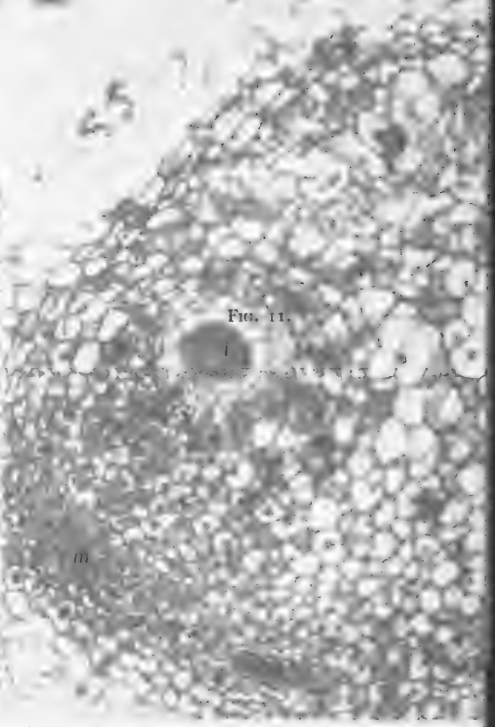


Fig. 11.

m

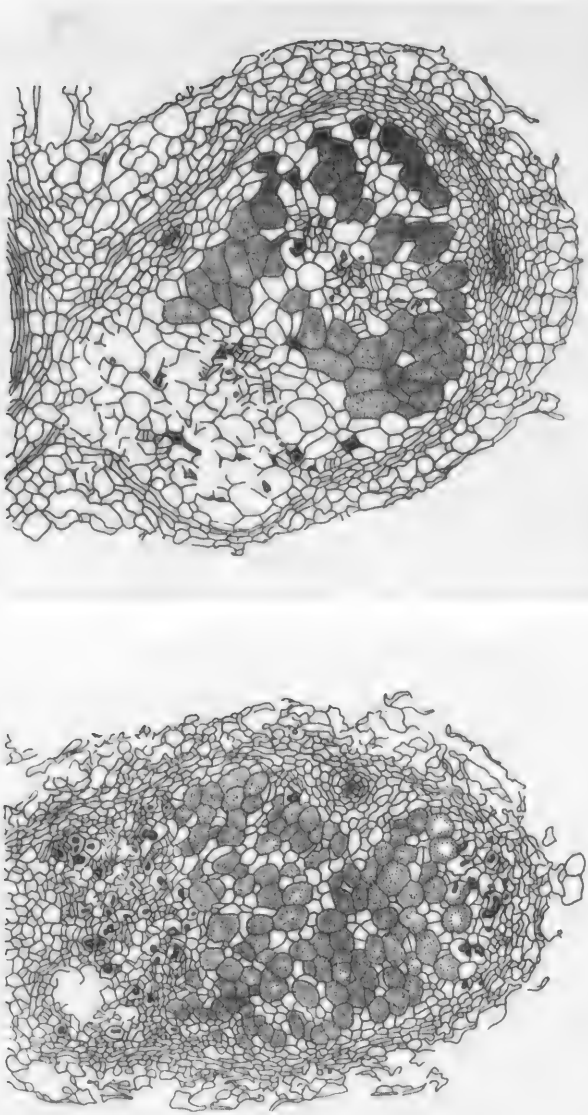
nodulation on the roots as shown in the previous paper (Nutman, 1957, plate I, fig. C). Normal or delayed effectiveness were not due to bacterial change and were thought to be due to the action of modifying factors in the host.

Inoculation of *ie* plants with strains such as ClF (known to be of outstanding effectiveness in the unselected host) gave a symbiosis which was largely effective. Wherever a fully effective response occurred in *ie* plants, the nodule structure was normal, the bacterial rods released from the infection threads giving rise to normal bacteroid tissue. Elsewhere nodules were of mixed structure consisting of parts which were normal and parts which showed tumorisation characteristic of *ie* ineffectiveness. The normal and tumorised regions were distributed as a mosaic in some nodules (text fig. 2*a*) ; in others, particularly in those plants showing a change in response and corresponding change in nodule pattern, the normal tissue was confined to the distal end of the nodule (text fig. 2*b*). Even with wholly ineffectively responding plants occasional isolated cells or small groups of cells escaped tumorisation and within these bacteroid formation took place.

6. DISCUSSION

Chen and Thornton (1940) ascribed ineffectiveness in symbiosis to the transient nature of the bacteroid containing tissue of the nodule and were able to show that the amount of nitrogen fixed was a direct function of the volume of bacterial tissue and its duration in time. This explanation was found also to apply to ineffectiveness due to boron deficiency where there is failure in vascular development as well as in bacteroid formation (Brenchley and Thornton, 1925). Again, in plants homozygous for the host factors i_1 or *ie*, ineffectiveness and bacteroid absence are correlated. Moreover the development of varying degrees of effectiveness in certain symbioses involving *ie* plants is closely related to the amount of bacteroid tissue present. These facts underline the primary role of the bacteroid in fixation.

From the point of view of development these results are of interest in showing that the sequence of changes leading to eventual bacteroid formation are determined by inherent factors in the host which operate in different ways and at different times. Thus the host gene *ie* acts in this sequence before the host gene i_1 and may cause a radical re-orientation in the development of the nodules. This takes place on contact between the bacteria and host cytoplasm and leads to formation of wound periderm presumably due to the production of some substance, possibly of a hormone nature. In this way the bacteria are confined in much the same way as are the pathogens in necrotic lesions produced by plant parasites (Gäumann, 1950, p. 456). This reaction of the host is very variable, depending upon the amount of development of bacterial rods taking place outside the infection thread. At one extreme the bacteria appear to be arrested in development immediately upon release into the cell and no divisions or other



TEXT-FIG. 2.—Cell wall tracings of *ie*/ClF nodules.

(*a*) A mixed response : both bacteroid-containing and tumorised tissue present in the same nodule.

(*b*) A changed response : tumorised tissue at the base and normal effective tissue at the tip of the nodule.

Released bacteria are shown as darkly shaded areas and bacteroid-containing cells are stippled.

changes ensue. With increasing bacterial proliferation the isolation of the infection takes place by an increasing number of secondary divisions of the original infected cell and its neighbours. With extensive secondary divisions the spindles become orientated radially to give rise to a spheroid periderm.

Under certain conditions the above *ie*/bacterial interactions do not take place and the symbiosis proceeds to completion. This appears to be determined by the genetic background in the host or by a change in the metabolism of the host either within the single nodule or progressively affecting the whole plant.

The irregular proliferation of the cells of *ie* nodules suggests a comparison with crown gall. Klein and Klein (1953) have demonstrated the close relationship between *Agrobacterium tumefaciens* and *Rhizobium* by transforming a strain of *Rhizobium* to an apparently normal virulent crown-gall producing organism by treatment with desoxyribonucleic acid from tumour-producing bacteria. In a later paper Klein (1954) summarised the known facts of crown-gall formation and concluded that wound substance, polymerised desoxyribonucleic acid produced by the bacteria, and auxin were all involved. In *ie* ineffectiveness the hypertrophy of the inner part of the nodule into a largely disorganised tumour-like growth, incapable of carrying out the symbiotic reactions of nitrogen fixation resembles crown gall to a remarkable degree. Even the normal root nodule may be regarded as an organised tumour of crown-gall type.

The i_1 gene acts at a later stage than *ie* and its effect is to prevent the final morphological stage in symbiosis—bacteroid formation. This gene inhibits bacteroid formation only with strain A, with other effective strains normal bacteroids are formed. Not only is the ineffectiveness due to the i_1 gene more specific than *ie* ineffectiveness but also it can only be revoked by specific mutation in the bacteria or by the action of supplementary host factors and in each case the reversal of the response in the host is complete.

As nothing is known of the nature of the stimulus leading to bacteroid formation no speculation can be offered regarding their absence in i_1 nodules. It is clear, however, that bacteroid formation is not conditioned solely by the host or by the bacteria.

7. SUMMARY

1. The structure of nodules produced by certain strains of *Rhizobium trifolii* on the roots of red clover plants homozygous for two independent recessive genes (i_1 and *ie*), has been examined. These genes cause failure in nitrogen fixation.

2. In one type (i_1) ineffectiveness is due to failure of the infecting bacteria to produce bacteroids ; this is found only with one particular strain of bacteria.

3. In the other type (*ie*) abnormal divisions of the host tissue take place in the vicinity of bacteria which have been released from infection

threads. This gives rise to a disorganised tumour-like growth in which no bacteroids are formed.

4. Tumorisation in *ie* nodules is not restricted to symbiosis with strain A, but occurs with a number of other effective strains of bacteria. With strains of bacteria exceptionally effective on unselected red clover varying degrees of effectiveness are found. These are associated with corresponding amounts of bacteroid formation. In certain nodules both tumorisation and normal bacteroid formation occur side by side.

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GENOTYPIC CONTROL OF CHROMOSOME BEHAVIOUR IN RYE

IV. THE ORIGIN OF NEW VARIATION

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1. INTRODUCTION

It is generally recognised that the pattern and the efficiency of chromosome behaviour within a population has been achieved under the influence of natural selection acting upon heritable variation arising in chromosome behaviour. This implies that new chromosome variation is continually being exposed, largely, it may be supposed, as the result of segregation and recombination of genes controlling the chromosomes. It is with the origin of such new variation that this investigation is mainly concerned.

In order to show experimentally that recombination can give rise to new forms of chromosome behaviour two homozygous inbred lines were crossed, and the derivatives of the hybrid were compared with the parents for evidence of new recombinant forms. A brief statement of some of the results has appeared in an earlier paper (Rees, 1955*b*). More complete data are now available and are discussed in the following account. While the main concern is with the origin of new forms of chromosome behaviour, the results have, at the same time, provided information about the nature of the gene systems controlling the chromosomes in heredity, in regard to their properties of recombination, and also about the way in which these genes act in controlling chromosome behaviour during development.

2. MATERIAL AND CHARACTERS

P_2 and P_5 (derived from the Swedish variety Stålråg) were the two inbred parental lines used. Both have been inbred by selfing for more than twenty generations, and as the following and other evidence indicates, they are virtually true breeding. Their cytology is described in detail elsewhere (Rees, 1955*a*). Two characters are dealt with (see plate I). They are :—

(1) *chiasma frequency*, expressed as the average per bivalent from scoring 20 p.m.c. in each plant.

(2) *pre-meiotic errors*. These result in p.m.c. containing extra nuclei or micro-nuclei whose chromosomes are frequently broken and invariably retarded at the early stages of meiosis. Usually less than 2 per cent. of cells in the affected plants are involved. In the parent lines they occur in P_5 but not in P_2.

A pedigree of the families derived from crossing P2 and P5 is represented in fig. 1. The scoring commenced with the F_3 generation —for two reasons. In the first place structural abnormalities such as pre-meiotic errors are generally rare or absent in the more heterozygous F_1 and F_2. These errors, and others which affect chromosome pairing, emerge with increasing unbalance of the more homozygous genotypes in later generations of inbreeding (fig. 2). In the second place, of course, family comparisons are possible from F_3 onwards, and where

TABLE 1

The chiasma frequencies in P2 and P5 over three years

1952		1953		1954	
P2	P5	P2	P5	P2	P5
1·79	1·41	1·54	1·64	1·90	1·66
2·01	1·54	1·68	1·70	1·91	1·69
2·04	1·69	1·86	1·74	1·94	1·74
2·04	1·74	1·87	1·77	1·96	1·76
2·08	1·76	1·88	1·79	1·98	1·79
				2·01	1·81

Item	SS	N	MS	VR	P
Years	0·0573	2	0·0287	2·70	0·10—0·05
Lines	0·3330	1	0·3330	31·42	<0·001
Years/lines . . .	0·1321	2	0·0661	6·24	<0·01
Error	0·2762	26	0·0106	...	...
Total . . .	0·7986	31	...	...	...

any differences occur between F_3 families and parents their heritability can subsequently be verified in the following two generations. Altogether more than 200 plants are involved.

3. CHIASMA FREQUENCY

For all families and lines the average frequencies for each year are given in fig. 3. The full data are not produced in order to save space.

Year to year variation. Although the parent lines are homozygous, the chiasma frequencies vary, sometimes considerably, between plants within lines grown in the same season. It has been shown that the *amount* of variation exhibited tends to be characteristic of a line and to differ between lines (Rees and Thompson, 1956). It is worth inquiring therefore to what extent the chiasma frequency of lines may vary from one season to the other, and whether any seasonal effect is the same for all lines. The complete data for P2 and P5 are given in table 1 ; and below is an analysis of variance based on these figures. The analysis shows that between lines the differences are significant, whereas between years they are not. It will be seen also that the interaction item for years and lines is significant. From this

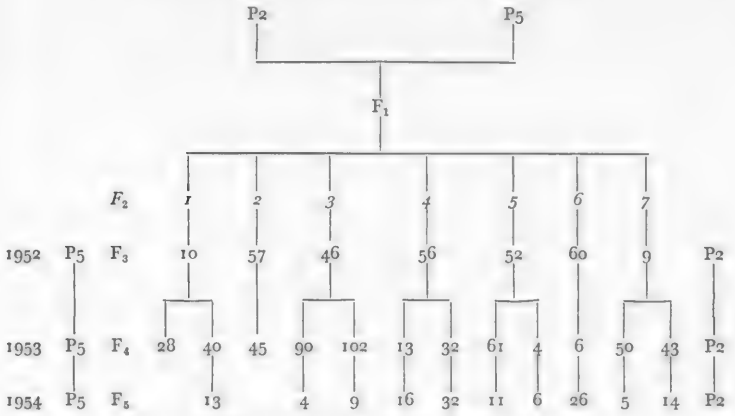

FIG. 1.—The history of the material. Scoring covers the three years from 1952 to 1954.

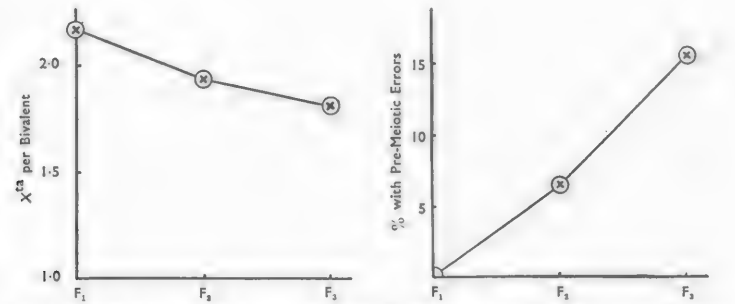

FIG. 2.—Graphs showing the average chiasma frequencies and the proportions of plants showing pre-meiotic errors in F_1, F_2 and F_3 generations from a cross between P2 and P5. With increasing homozygosis the chiasma frequency falls and the proportion of plants showing pre-meiotic errors rises.

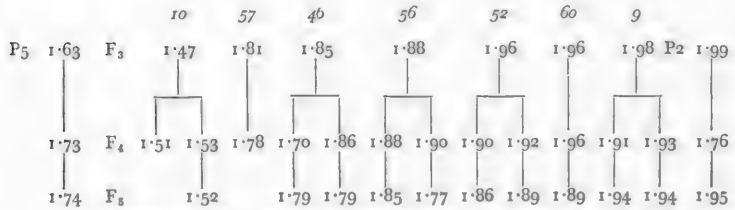

FIG. 3.—The mean chiasma frequencies of the families and lines. The order of families is the same as in fig. 1.

it is concluded that the response to different growing conditions from year to year is not the same for the two lines, P2 in this case being more variable between years.

Segregation and recombination. The differences in chiasma frequencies between families within F_3, F_4 and F_5 are highly significant, the probability values being less than 0·001 in each instance. Regression

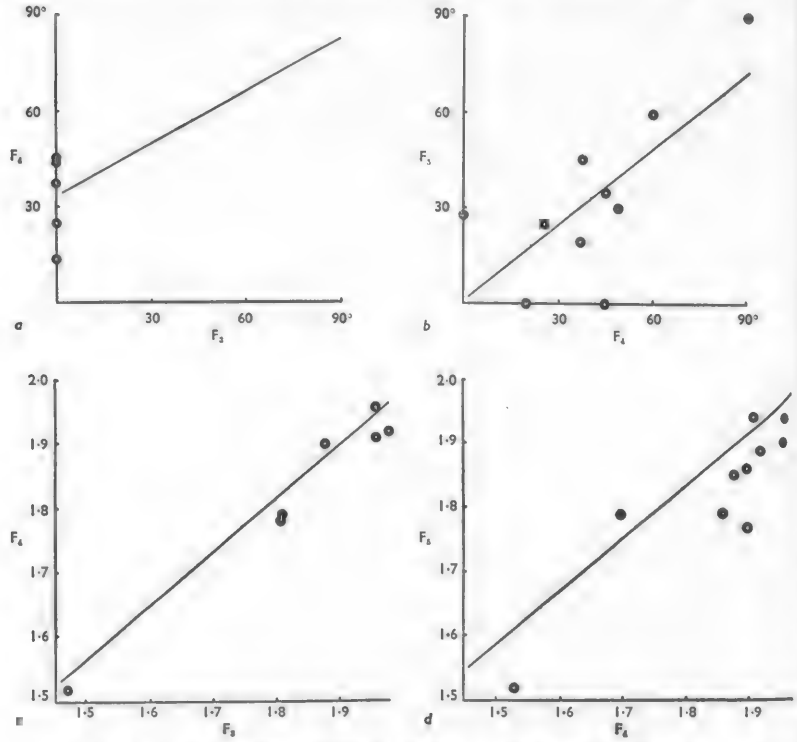

FIG. 4.—*a*, *b*. Graphs showing regressions between the proportions (in angles) of plants with pre-meiotic errors in related families in F_3, F_4 and F_5 respectively. *c*, *d*. The regressions for the mean chiasma frequencies of the families.

analyses between parent and offspring families, F_3 and F_4, F_4 and F_5, prove that these differences are genotypically controlled. The regressions (fig. 4) are significant at the 0·001 level.*

Family 10 and its derivatives are of particular interest in that, considered over the three years, they have significantly lower chiasma frequencies than either parent. These families with chiasma frequencies transgressing the parental values must have arisen as indicated previously (Rees, 1955*b*) by recombination. Equally evident from

* For calculating the regression between F_3 and F_4 families the data for sister families in F_4 were pooled.

fig. 3 is the wide range of chiasma frequencies in the families which have resulted from segregation and recombination. The values make up a more or less continuous series, from lower than the "low" parent to as high as the "high" parent.

4. PRE-MEIOTIC ERRORS

The expression of this character within anthers is entirely different from chiasma frequency and is such that the abnormal and normal cells develop side by side. Furthermore in some anthers all the p.m.c. are normal while in other anthers of the same plant some p.m.c. are abnormal. Consequently even in the homozygous parent P_5 not all the plants are scored as showing the abnormality. For purposes of comparison therefore the measure adopted for pre-meiotic errors has been the proportion of plants affected in the line or family. These proportions were then transformed to angles for statistical analyses. The angular values are presented in fig. 5.

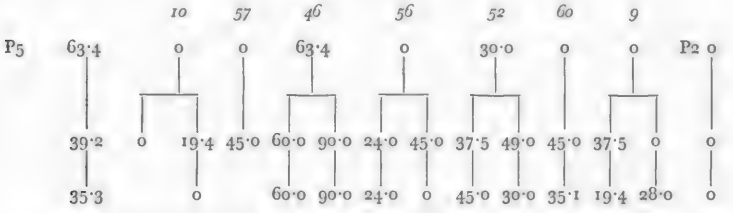

FIG. 5.—The proportions (as angular values) of plants in lines and families showing pre-meiotic errors.

Firstly, no significant difference was found between inbred P_5 plants grown in different years. Secondly, it will be seen that a whole range of differing families appear in the derivatives of the P_2/P_5 cross. As for chiasma frequency, regression analyses between parent and offspring families confirm the heritability of these differences (see fig. 4). Again as for chiasma frequency one sub-line, *viz.* family (46) and its derivatives, are of particular interest from the point of view of recombination and the origin of new chromosome variation. In these families the proportion of plants which show pre-meiotic errors is significantly higher than in the affected P_5 parents over the same period ($P = 0{\cdot}05$). Evidently the new variation has arisen by recombination of the genes affecting the penetrance of the pre-meiotic errors.

That family 46 and its derivatives represent entirely new forms is confirmed when these are compared with P_5 in respect of the *proportions of abnormal p.m.c.* within anthers, *i.e.* in terms of expressivity. The proportions have in fact been calculated for all plants from the scoring of about 200 cells in each. As percentages the family averages for

affected plants appear in fig. 6. For the parent P_5 the average is about 1 per cent. in each of the three years, 2 per cent. being the highest value for any one plant. In contrast families descended from 46 have plant averages of up to 4 per cent. abnormal cells, with 8 and 9 per cent. abnormal in some F_4 and F_5 individuals. Using the percentages (transformation makes virtually no difference to such small values) a regression between F_4 families and those related to them in F_5 is significant at the 5 per cent. level ; and thereby establishes heritability of the degree of the abnormality expressed within anthers.

In anthers where the relative frequency of abnormal p.m.c. is very high it is clear even in squash preparations that the abnormal cells tend to be localised in groups, but exactly where in the anthers these groups occur is not known. Comparable examples of localisation of pre-meiotic errors are reported by Darlington and Haque (1955), and also by Holden and Mota (1956).

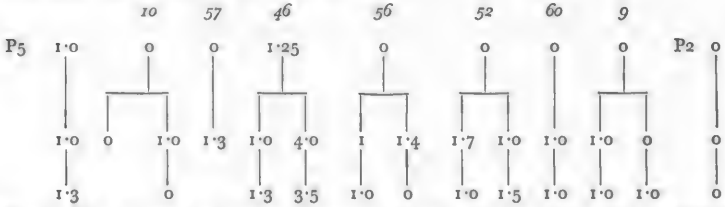

FIG. 6.—The average percentages of p.m.c. showing pre-meiotic errors in the affected plants in lines and families. Note the relatively high values in *46* and its descendants.

5. THE TWO CHARACTERS

Recombination so far has been considered only in respect of genes affecting the two characters separately. For chiasma frequency and also for pre-meiotic errors new variants were shown to arise through recombination. When the two characters are considered together, as in fig. 7, it is seen that a wide range of forms emerges, and families with high or low chiasma frequencies turn up, either with or without pre-meiotic errors in p.m.c. There is thus no doubt of the recombination of the genes controlling the two characters in a number of ways. It also would appear from the graph that these two particular characters are inherited independently of one another.

6. DISCUSSION

The expectation of discovering new chromosome variation resulting from gene recombination has been fully justified by the results described. These, in conjunction with earlier evidence, *e.g.* Maeda (1937) and Peto (1933) enable us to visualise how natural selection acting upon the variation arising in this way determines the behaviour pattern of the chromosomes in a population. This pattern we might expect to vary adaptively from one environment to another. As yet,

unfortunately, not much information is available concerning such variation in wild populations within species. But what information there is amply confirms this (*cf.* Darlington, 1956).

From a developmental point of view it may be noted that the two characters dealt with show variation even between the p.m.c. of the same anther, but they do so in very different ways. For chiasma frequency there is no sharp distinction between cells with few and

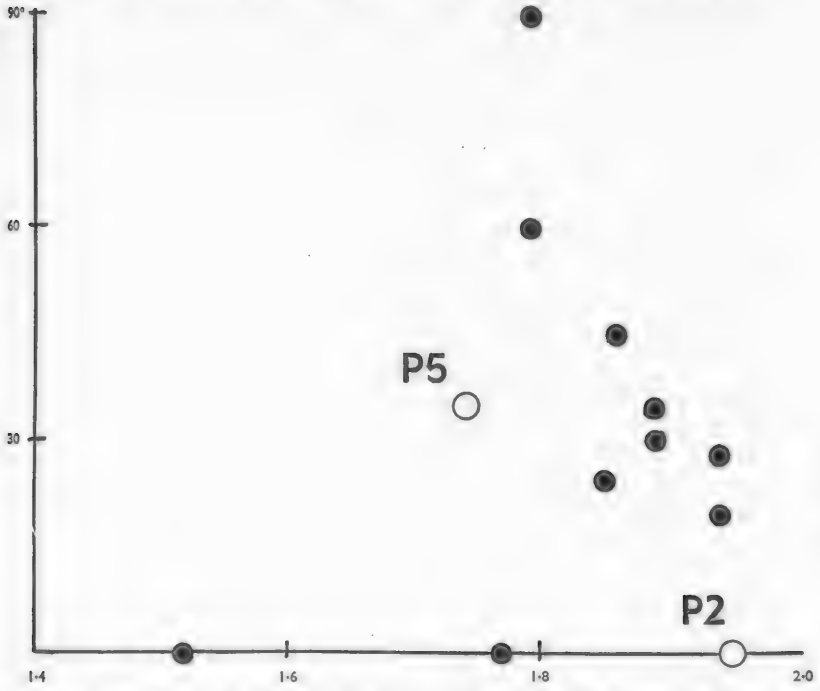

FIG. 7.—Proportions (in angles on the vertical scale) of plants in F_3 families with pre-meiotic errors plotted against mean chiasma frequencies of the families. Parent lines for the same year are included. Note the wide range of types in F_3 resulting from segregation and recombination. The two characters appear to be independent in inheritance.

many chiasmata, and whether the plant frequency is high or low the p.m.c. tissues are qualitatively more or less homogeneous. At the same time there is a range of p.m.c. chiasma frequencies within an anther, thereby causing, in respect of the amount of recombination, a variety in the gametes produced. This variety, it has been shown, is genetically controlled (Rees and Thompson, 1956).

Variety with regard to pre-meiotic errors within anthers is of an entirely different kind. Two sharply distinguished types of cells develop side by side and where increased expressivity of the character was achieved this resulted in even more pronounced qualitative

heterogeneity of the p.m.c. tissue. Such a divergence in development, *e.g.* in P_5, in what normally constitutes a homogeneous tissue, is instructive from the point of view of development generally. As Darlington and Haque (1955) have pointed out with reference to a comparable case in *Allium*, the development of the two kinds of p.m.c. represents, in simple form, the problem of differentiation. When viewed from the standpoint of normal development of a simple or complex organ, divergence between cell types is the first essential, accomplished no doubt as the above authors suggest with the aid of diffusion barriers between the different kinds of cells. The second essential is that the components of the organ, being complementary to one another, are in such proportions as to confer, collectively, greatest efficiency to the organ and to the phenotype generally. Consequently the differentiation during development of the organ is achieved in such a way, or at such a time, that the tissues comprise well defined fractions of the whole. The achievement of the right proportions, it may be assumed, is the result of natural selection, which of course implies that the tissue proportions are genetically determined and show heritable variation. The rye results presented above show, albeit in connection with an abnormal organ, how this heritable variation in the relative amounts of the component tissues can arise by recombination. In the parental line P_5 it is seen that one class of cells comprises 1 per cent., whereas in the F_4 and F_5 derivatives this class constitutes up to 9 per cent. of p.m.c. Thus, at cell level, exactly the same capacity of adjustment in developmental pathways is indicated as has been demonstrated for more elaborate levels of organisation, *e.g.* the symmetry of eyes and other body parts in *Drosophila* (*cf.* Waddington, 1942, Mather, 1953).

7. SUMMARY

1. A cross was made between two inbred lines of rye differing in two characters, (*a*) chiasma frequency, and (*b*) a structural abnormality resulting from pre-meiotic errors. In the F_3 and later derivatives of the hybrid between the lines, recombination was demonstrated for genes controlling chiasma frequency, and for genes affecting the penetrance and expressivity of pre-meiotic errors. Recombination is also demonstrated for the two characters considered jointly, the results indicating they are inherited independently.

2. The effect of year to year variation in chiasma frequency is not the same for the two inbred lines.

3. Heritable variation in the proportions of p.m.c. showing pre-meiotic errors indicates how, in normal organs, the relative amounts of the component tissues may be adjusted under natural selection.

Acknowledgment.—I am grateful to Mr J. B. Thompson for his valuable assistance with this work.

Plate

1. The result, at MI, of pre-meiotic errors. Seven normal bivalents, extra bivalents, univalents and fragments.

2, 3. MI in p.m.c.'s of an " asynaptic " family derived by recombination of genes controlling chiasma formation. Only two bivalents in 2, none in 3.

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HAPLOIDS AND POLYHAPLOIDS IN *ÆGILOPS* AND *TRITICUM*

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1. INTRODUCTION

THE examination of chromosome behaviour in haploids and polyhaploids is of importance for two reasons. First, the amount of bivalent formation at meiosis is a measure of the level of chromosome structural duplication in the species from which the haploid was derived. Many investigators have drawn conclusions about the phylogenetic relationships of species from the meiotic pairing in hybrids between them. In such work, however, it is necessary to be aware, as has been shown by Stebbins and Pun (1953), how much of the pairing is autosyndetic. The most useful measure of the amount of autosyndesis is provided by records of structural duplication within species obtained from studies of their haploids. Further, knowledge of the extent of structural duplication in species at different chromosome levels may be a valuable aid in understanding the processes of evolution by allopolyploidy, especially in relation to the theory of increasing differentiation between initially semi-homologous chromosome sets proposed by Darlington (1937). Secondly, meiosis in haploids, particularly of wheat, may show how general are the relationships between bivalents and the two types of univalent associations recently described by Person (1955) in a polyhaploid *Triticum vulgare*. In this plant it was shown that the parallel, side-by-side, association of univalents at metaphase, widely described elsewhere, was a function of their homology, but that end-to-end association of univalents was not due to homology.

2. ORIGINS OF THE HAPLOIDS

A haploid plant of *Ægilops longissima*, a polyhaploid of *Triticum timopheevi*, and two polyhaploids of *Triticum vulgare* have been studied. Their origins are described below.

(1) Ægilops longissima

Amongst the progeny of the cross *Ægilops longissima* Schwein. et Musch. ($2n = 14$) × *Triticum durum* Desf. var. *cærulescens* Körn. ($2n = 28$) there was one plant which was morphologically like the seed parent and was at first thought to be a self. When it was noted that its anthers were non-dehiscent and that the glumes gaped open at flowering the plant was examined cytologically and it was found to have only seven chromosomes. It was, therefore, a haploid *A. longissima* which had arisen parthenogenetically. It set no seed.

(ii) Triticum timopheevi

One seed with twin embryos was produced in the cross *Triticum timopheevi* Zhuk. var. *typicum* Zhuk. ($2n = 28$) × *Triticum monococcum* L. gr. *flavescens* Körn. ($2n = 14$). Both embryos developed, one formed a plant, with 21 chromosomes, which was morphologically similar to other hybrids of the same parentage. The other embryo formed a plant which was morphologically identical with *T. timopheevi* though somewhat less vigorous, and with only 14 chromosomes. This plant, which was therefore polyhaploid *T. timopheevi*, was colchicined and produced some grains which gave rise to 28-chromosome progeny. There were, however, also some undoubled tillers available for cytological examination.

(iii) Triticum vulgare

Two types of polyhaploid *Triticum vulgare* Vill. var. Holdfast ($2n = 42$), = *Triticum æstivum* L., were available. One was found in the progeny of a 45-chromosome plant which had the full complement of *T. vulgare* plus three *Secale cereale* chromosomes. At meiosis in the parent there were always 21 bivalents and 3 univalents, and in its progeny, apart from the polyhaploid, there were no plants with less than 42 chromosomes and none which at meiosis formed fewer than 21 bivalents. It is, therefore, most unlikely that the 21-chromosome plant which occurred in the progeny was any other than normal polyhaploid *T. vulgare*. This was confirmed by the phenotype of the plant which was identical with that of a normal Holdfast plant, except for the effects of complete sterility on the spikes, and for reduced vigour.

In the progeny of a 44-chromosome (rye chromosome II disomic addition) plant of *T. vulgare* variety Holdfast (Chapman and Riley, 1955) there was a plant with 22 chromosomes. This plant presumably arose parthenogenetically following a normal reduction in disomic addition II, and would then possess the polyhaploid complement of *T. vulgare* plus one rye chromosome II. The status of this plant may be represented by the formula 3xT+1S. The presence of the rye chromosome follows from the cytological results and from the phenotype of the plant which was characteristic of Holdfast plus rye chromosome II : with short flag leaves, considerable mildew resistance, tapering ears and lengthened lemma points at the apex of the ear. A few grains were obtained from crossing this plant with normal Holdfast plants. These grains were red skinned as are those of disomic addition rye II, although the grain of Holdfast is white.

(iv) Ægilops longissima×Triticum timopheevi

One hybrid, derived from two of the forms of which haploids were available, was examined. This was the F_1 of the cross *Ægilops longissima* Schwein. et Musch. ($2n = 14$) × *Triticum timopheevi* Zhuk. var. *typicum* Zhuk. ($2n = 28$).

3. NOTE ON TERMINOLOGY

In the present material, as in that of earlier workers on haploids and polyhaploids in the Triticinae (Gaines and Aase, 1926 ; Krishnaswamy, 1939, McGinnis and Unrau, 1952, and Person, 1955), it was difficult to distinguish first metaphase of meiosis from first anaphase. This was due to the absence of a clear metaphase plate in cells with univalents only, the arrangement of univalents at the poles and the non-synchronous separation of bivalents when a limited number were present. It is therefore proposed to use the term *metaphase* to apply to the period of division which commenced when the body repulsions of the chromosomes were overcome and the bivalents were orientated on the plate and the univalents at the poles. In the present sense *metaphase* will be considered to have ended by the time that the chromosomes at the poles had opened-out, except at the centromeres, into double strand X shapes. From this time until interphase will be termed *telophase*.

The segmental similarity of non-partner chromosomes of a species which results in the formation of bivalents in its haploids or polyhaploids will be referred to as *structural duplication.*

The 22-chromosome plant (3xT+1S) will be termed *aneupolyhaploid T. vulgare.*

4. METHODS

Chromosome counts were made on squashes of root-tips pre-treated with α-bromonaphthalene, fixed in glacial acetic acid, and stained by the Feulgen technique. Meiosis was examined on permanent Feulgen squashes of pollen mother cells of anthers fixed in acetic-alcohol.

5. OBSERVATIONS

(I) *Chromosome associations*

By the commencement of metaphase in all the plants under consideration in addition to associations dependent upon chiasmata—bivalents and trivalents—there were two main types of univalent associations. These may be called, following the terminology of Person (1955), side-by-side (s-s) and end-to-end (e-e) associations.

In s-s associations two univalents lay alongside and parallel to each other (plate, fig. 5) and sometimes appeared to be connected. Occasionally the members of s-s associations diverged from each other at one end, and often they were similarly flexed, the convex side of one lying within the concave side of the other (plate, fig. 2). The s-s pairs were usually in the polar groups of univalents where they orientated at random, but occasionally they occurred at the equator always at right angles to the polar axis (plate, fig. 5). Univalents which appeared to have taken part in s-s pairing were sometimes seen at equivalent positions on either side of the equator (plate, fig. 3) as though the chromosome halves of the association had just separated.

The members of e-e associations lay in tandem, and sometimes the ends were clearly connected by a single chromatin thread (plate, fig. 5). These associations were generally at the poles (plate, fig. 2) where they were orientated at random. Those at the equator usually lay along (plate, fig. 5), and rarely at right angles (plate, figs. 1 and 4) to, the plane of division. There were occasional multi-e-e associations in the two *T. vulgare* polyhaploids and in the *A. longissima*× *T. timopheevi* hybrid, but usually only two univalents were paired. The maximum number of univalents involved in multiple e-e's was five.

A few end-to-side pairs were observed in which the end of one univalent was connected by a staining thread to the centre of another (plate, fig. 3). Very rarely mixed multiple associations occurred combining various arrangements of s-s, e-e and end-to-side associations (plate, figs. 4 and 6).

The mean frequencies of bivalents, trivalents, and s-s and e-e associations, and the frequency distributions of bivalents are recorded

in table 1. Usually there was one terminalised chiasma per bivalent, although in the aneupolyhaploid *T. vulgare* and in the *A. longissima* × *T. timopheevi* hybrid there were a few bivalents with two chiasmata. The trivalents were V-shaped chains with adjacent chromosomes joined by one chiasma. Trivalents were found only where inter- and intra-genome homologies were possible in the polyhaploids and never in the haploid of the diploid *A. longissima*. There were never more than three bivalents in cells with a trivalent in the *T. vulgare* polyhaploids and the hybrid, and no bivalents in cells with a trivalent in polyhaploid *T. timopheevi*.

In order to determine the relationships between the three types of association, chiasmata, s-s, and e-e, the correlation coefficients between the numbers of chromosomes involved in each have been estimated (table 2). The estimates were based on the numbers of chromosomes rather than on the numbers of associations in order to accommodate associations in which more than two chromosomes were sometimes involved : trivalents and multiple e-e's.

The key to this set of data is the pattern of correlations found in polyhaploid *T. vulgare*. In this plant there were significant negative correlations between the numbers of chromosomes in s-s and chiasmata associations and between the numbers in s-s and e-e associations. Although the pattern of correlations is less marked in the other plants there is none which reverse the relationships in polyhaploid *T. vulgare*. Indeed in the *A. longissima* × *T. timopheevi* hybrid the correlations were all in the same direction as in that plant, although only the coefficient between s-s and e-e was significant. These data may be interpreted to mean that chromosomes which frequently participate in chiasmata associations may also, on different occasions, be found in s-s associations. On the other hand chromosomes which are frequently found in e-e associations may sometimes participate in s-s but rarely in chiasmata associations.

It has not been shown, despite the detailed investigation of the problem by Levan (1942) that chiasma formation can take place between non-homologous regions of chromosomes. Therefore the presence of chiasmata associations in this material may be accepted as evidence of some segmental correspondence. Thus the significant correlation between s-s and chiasmata associations must imply that the formation of s-s associations was also due to the segmental homology of the partners. However, the non-significant correlation between chiasmata and e-e associations provides no evidence of the homologous or non-homologous status of this type of pairing.

Further evidence regarding the significance of the univalent associations may be obtained from a comparison of the 21-chromosome polyhaploid and the 22-chromosome aneupolyhaploid *T. vulgare* plants. In the aneupolyhaploid there was a significantly higher mean number of univalents per cell (table 1) than in the polyhaploid ($t_{=(142)} = 3{\cdot}52$, $P = <0{\cdot}001$), whereas the means of bivalents per

TABLE 1

Bivalent frequency and mean pairing at metaphase

Plant	No. cells	Bivalent frequency (cells)						Mean pairing				
		0	1	2	3	4	5	univ.	biv.	triv.	s-s	e-e
polyhaploid *T. vulgare* ($3x = 21$)	104	0·15	0·45	0·28	0·10	0·02	...	18·05±*0·19*	1·38±*0·09*	0·07	1·32±*0·12*	0·75±*0·07*
aneupolyhaploid *T. vulgare* ($3x+1 = 22$)	40	0·17	0·47	0·27	0·09	...	...	19·28±*0·29*	1·25±*0·13*	0·08	1·73±*0·17*	1·15±*0·14*
polyhaploid *T. timopheevi* ($2x = 14$)	60	0·77	0·21	0·02	...	...	...	13·45±*0·13*	0·25±*0·06*	0·02	0·45±*0·08*	0·32±*0·07*
haploid *A. longissima* ($1x = 7$)	100	0·89	0·09	0·02	...	...	...	6·74±*0·25*	0·13±*0·12*	0·00	0·21±*0·13*	0·15±*0·11*
A. longissima × *T. timopheevi* ($3x = 21$)	75	0·21	0·37	0·26	0·08	0·01	0·01	17·99±*0·28*	1·43±*0·13*	0·05	0·53±*0·07*	0·51±*0·06*

TABLE 2

Correlation coefficients between the numbers of chromosomes in different types of associations

Plant	d.f.	Xta.-s-s			Xta.-e-e			s-s-e-e		
		n	*t*	P	*n*	*t*	P	*n*	*t*	P
polyhaploid *T. vulgare* ($3x = 21$)	102	−0·2810	2·63	0·02-0·01	0·1184	1·21	0·2-0·1	−0·2202	2·01	0·05-0·02
aneupolyhaploid *T. vulgare* ($3x+1 = 22$)	38	−0·0293	0·18	0·9-0·8	−0·1384	0·86	0·4-0·3	−0·5332	3·89	<0·001
polyhaploid *T. timopheevi* ($2x = 14$)	58	−0·1451	1·12	0·3-0·2	−0·0851	0·65	0·6-0·5	−0·0279	0·21	0·9-0·8
haploid *A. longissima* ($1x = 7$)	98	−0·0474	0·47	0·7-0·6	−0·0703	0·70	0·5-0·4	−0·1478	1·48	0·2-0·1
A. longissima × *T. timopheevi* ($3x = 21$)	73	−0·1707	1·48	0·2-0·1	0·2019	1·76	0·1-0·05	−0·2241	1·91	0·05

cell were not significantly different ($t_{(142)} = 0{\cdot}78$, $P = 0{\cdot}5$-$0{\cdot}4$). Indeed frequencies of cells with from 0-3 bivalents, and with trivalents, were strikingly similar in these plants. The extra chromosome in the aneupolyhaploid was thus behaving as a univalent and not affecting the numbers of associations with chiasmata. It was, therefore, not homologous with any of the chromosomes of the wheat complement. This confirms the impression, formed by the phenotype of the plant, that the extra chromosome was rye chromosome II. In the aneupolyhaploid the mean number of s-s associations (table 1) was not significantly higher than that in the polyhaploid *T. vulgare* ($t_{(142)} = 1{\cdot}89$, $P = 0{\cdot}1$-$0{\cdot}05$) whereas there was a significant increase in the number of e-e associations ($t_{(142)} = 2{\cdot}57$, $P = 0{\cdot}02$-$0{\cdot}01$). Since the number of e-e associations may be increased by the presence of an additional non-homologous chromosome this type of pairing cannot be dependent on structural similarity.

Thus participation in s-s associations is between segmentally homologous partners. In contrast e-e associations are formed between non-homologous chromosomes.

(ii) *Univalent distributions*

In the majority of cells in the three *Triticum* polyhaploids and in haploid *A. longissima* most univalents had moved to the poles by metaphase (plate, figs. 1, 3 and 6). However, in the polyhaploid *T. vulgare* plants there were from 0-5, and in the polyhaploid *T. timopheevi* from 0-4, univalents left at the equator (plate, figs. 2, 3, 5 and 6). These univalents were generally orientated parallel to the equator but occasionally lay along the polar axis. However, those at the poles were dispersed at random to the plane of division and to each other, except where they were involved in s-s or e-e associations.

The segregation of univalents to the poles has been compared with the calculated random distribution in polyhaploid *T. vulgare*, polyhaploid *T. timopheevi* and haploid *A. longissima*. A varying number of chromosomes were excluded from the polar groups because of their participation in bivalents or trivalents, or because they remained at the equator as univalents. The number segregating was thus not always the full complement, but ranged for example in polyhaploid *T. vulgare* from 10 to 21. In order to overcome this difficulty in calculation, the ratio at the poles for each cell was so adjusted that its sum was the haploid number concerned. This was achieved, for example, in polyhaploid *T. vulgare* by multiplying each side of the ratio by the proportion by which 21 exceeded the actual number segregating. Thus in a cell in which 6 chromosomes remained at the plate and the others segregated to the poles in the ratio 10 : 5 the adjusted ratio became 14 : 7. The ratio 6 : 8 became 9 : 12 and so on. Where the adjusted ratio did not involve whole numbers it was taken to the nearest whole number. An advantage of this method

is that no assumptions were made concerning the behaviour of the chromosomes at the equator.

The segregation of univalents to the poles at metaphase was not significantly different from random in polyhaploid *T. vulgare* ($\Sigma\chi_{(4)} = 5{\cdot}27$, $P = 0{\cdot}3$-$0{\cdot}2$) and in haploid *A. longissima* ($\Sigma\chi_{(2)} = 2{\cdot}11$, $P = 0{\cdot}5$-$0{\cdot}3$). In polyhaploid *T. timopheevi*, however, there was a significant divergence from random (table 3) largely due to an excess in the 6-8 class and a deficiency in the 5-9 class. Random segregation of univalents in Triticinæ haploids have been reported in *T. monococcum* (Chizaki, 1934), *T. durum* (Kihara, 1936), *T. vulgare* (Yamamoto,

TABLE 3

Segregation of univalents to the poles at metaphase in polyhaploid T. timopheevi

Class	Cells observed	Random		Deviation		χ^2
7-7	11	9·84		1·16		*0·14*
6-8	26	17·23		8·77		*4·46*
5-9	6	11·48		5·48		*2·56*
4-10	4	5·74		1·74		
3-11	0	2·09		2·09		
2-12	0	0·53	8·44	0·53	4·44	*2·34*
1-13	0	0·08		0·08		
0-14	0	0·00		0·00		
Total . .	47	47·00		...		9·50

$\Sigma\chi^2_{(3)} = 9{\cdot}50$ $P = 0{\cdot}05$—$0{\cdot}02$.
The bracketed data were summed to bring the expected to more than 5·0.

1936) and *A. ovata* (Matsumura, 1940) and excessive regularity in *T. monococcum* (Katayama, 1935) and *T. vulgare* (McGinnis and Unrau, 1952 and Person, 1955). Excessive regularity may be due to the disjunctional separation of s-s associations at early metaphase proposed by Person and observed to occur in the present material (plate, fig. 3). Differences in the behaviour of different haploids, even in the same species, might then be conditioned by genetically or environmentally determined variations in the numbers of s-s associations.

In the 40 cells which it was possible to analyse at telophase in polyhaploid *T. vulgare* the mean number of univalent laggards was $4{\cdot}75 \pm 0{\cdot}19$. This is significantly greater than the mean number of univalents ($1{\cdot}22 \pm 0{\cdot}19$) at the equator at metaphase ($t_{(98)} = 12{\cdot}74$, $P = <0{\cdot}001$). There is therefore confirmation for observations of Person that some univalents move from the poles to the equator at the end of metaphase in *T. vulgare* polyhaploids.

The ratios of complete chromosomes at the poles at telophase in this plant were adjusted to bring their sums to 21, as with metaphase ratios. The frequency distribution of telophase segregations did not

differ from random ($\Sigma\chi_{(3)} = 3{\cdot}27$, P = 0·5-0·3) or from segregations at metaphase ($\Sigma\chi_{(3)} = 6{\cdot}06$, P = 0·1-0·05). The increased number of univalents at the equator had therefore resulted from movements from either pole at random.

6. DISCUSSION

(I) *Chromosome behaviour*

The end-to-end pairing of univalents has previously been reported in *Triticum* haploids (Chizaki, 1934, fig. 10; Katayama, 1935; Krishnaswamy, 1939; McGinnis and Unrau, 1952, and Person, 1955). The present results confirm those of Person that these associations do not depend upon homology. Person inclined to Ribbands' (1937) view that they arose from accidents in positioning. This seems unlikely since the ends of the paired univalents are often connected by a staining thread (plate, fig. 5). However, these connections closely resemble those demonstrated by Thomas and Revell (1946) to join secondarily paired bivalents at metaphase in *Citer.* In this material it was shown that the secondary pairings of bivalents was due to the fusion, during pachytene, of regions of heterochromatin of chromosomes already homologously paired. The similarity in the appearance of the connections suggests that the end-to-end association of univalents in *Triticum* may also be due to the fusion of heterochromatin, as was originally proposed by Kostoff (1938). It can be demonstrated, by cold treatment, that there are some chromosomes with terminal and some with intercalary regions of heterochromatin in *T. vulgare.*

End-to-side pairing was much less frequent than end-to-end. If, therefore, both types were due to connections of heterochromatin then the fusion of terminal with terminal segments was much more frequent than the fusion of terminal with intercalary segments.

The general conclusion from the previous work (Richardson, 1935; Ribbands, 1937; Östergren and Vigfusson, 1953; Person, 1955) has been, as at present, that the side-to-side pairing of univalents was a function of their homology. The observations of Richardson, Ribbands, and Östergren and Vigfusson showed that the metaphase association was preceeded by zygotene pairing, but that chiasma-formation failed. Most authors have believed that the continued pairing was due to secondary association, made possible by the proximity of the partners as a result of coiling or positional correlation. Östergren and Vigfusson considered the continuance of pairing to metaphase in asynaptic rye to be due to stickiness. Since, however, the secondary association of bivalents is due to the fusion of heterochromatic regions it seems possible that secondary association between s-s partners has a similar cause. If homologous pairing, without chiasma-formation, at prophase were followed by the fusion of more than one, or of one long, region of heterochromatin of the partners then the parallel alignment would be maintained.

Therefore, on the basis of the hypothesis of heterochromatin fusion, side-by-side, end-to-end and the rare end-to-side associations would all have the same origin. The distinction between them would arise because the fusions in end-to-end and end-to-side pairs had occurred between segments of chromosomes which lay adjacent by chance. Whereas the fusions in side-by-side pairs had occurred between regions of chromosomes already homologously paired. Mixed multiple associations must then have resulted from various combinations of fusions of terminal with terminal, intercalary with intercalary and intercalary with terminal segments.

(ii) *Structural duplication in polyploids*

The bivalent-univalent frequency in haploid *A. longissima* was of much the same order as that reported in haploid *T. monococcum* (Kihara and Katayama, 1933; Chizaki, 1934; Smith, 1946). Further, the bivalent frequency in haploid *A. longissima* was in the same relationship to that reported for the polyhaploid of a tetraploid *Ægilops* species, *A. ovata* (Matsumura, 1940), as was that in *T. monococcum* to the frequency in the polyhaploid of *T. durum* (Kihara, 1936). The frequency in the polyhaploids of the tetraploids *T. timopheevi*, *T. durum*, and *A. ovata* was essentially similar. Finally, the pairing in the present polyhaploid of *T. vulgare* falls within the range previously described in polyhaploids of hexaploid wheats (Gaines and Aase, 1926; Yamamoto, 1936; Raw, 1937; Krishnaswamy, 1939; Person, 1955).

TABLE 4

Mean proportion of complement as univalents, bivalents and trivalents in a haploid and two polyhaploids

Species	univalents	bivalents	trivalents
A. longissima ($1x = 7$) .	0·96	0·04	0·00
T. timopheevi ($2x = 14$) .	0·96	0·04	0·00
T. vulgare ($3x = 21$) . .	0·86	0·13	0·01

The three examples which are at present available may therefore be accepted as typical of the behaviour of haploids of species at the diploid, tetraploid and hexaploid levels in the closely related genera *Triticum* and *Ægilops*. The amount of pairing in haploids may be used as a measure of the level of structural duplication in the species concerned. When the amount of pairing is considered as a proportion of the chromosome number (table 4) it is found that there is relatively no more structural duplication in the tetraploid *T. timopheevi* than in the diploid *A. longissima*. However, *T. vulgare* has more than three times as much duplication as the diploid and the tetraploid.

The two species contributing to the tetraploid may have possessed karyotypes within which there was about the same amount of duplication as in *A. longissima* or *T. monococcum* but between which there were no similarities. Whereas the third genome added in the origin of the hexaploids may have had some homology with either, or both, genomes of the tetraploid. That is, the differences may be residual from the original structural condition at the establishment of the polyploid species.

Alternatively, however, Darlington (1937) has suggested that there may be selection for those structural alterations which increase the differentiation of the chromosome sets of polyploids, since the multivalents which resulted from duplications would lead to reduced fertility and genetic instability. If selection favoured cytological "diploidisation" in allopolyploids, through a reduction in the level of structural duplication, it might be expected that more duplication would remain in the younger polyploids. Therefore the present results may mean that there has been more divergence between the two chromosome sets of the tetraploid species than between the third and the original two sets of the younger, hexaploid, species.

The accounts of polyhaploids in hexaploid wheats indicate pairing in excess of that in the synthetic polyhaploids produced as hybrids between the putative parents (McFadden and Sears, 1946; Kihara and Lilienfeld, 1949). It is unlikely, therefore, that there has been any decrease in the structural duplication at this level. The synthetic polyhaploid of a tetraploid wheat has not been produced so that a similar comparison is not possible at this level. However, polyhaploid *A. ovata* (Matsumura, 1940) has considerably less pairing than the hybrids between its putative parents (Kihara, 1937; Lilienfeld, 1951). The development of cytological "diploidisation" in *A. ovata* must strengthen the probability that a similar process is responsible for the very different amounts of structural duplication in species of different levels of polyploidy in *Triticum*.

(iii) *Pairing in haploids and hybrids*

One of the difficulties in the interpretation of synaptic pairing in hybrids is the possibility that some pairing may be autosyndetic. This has been shown by Stebbins and Pun (1953) to have been responsible for some mistaken diagnoses with regard to the genome relationships between *Triticum* and *Agropyron*. It is therefore desirable to know the amount of structural duplication in the parents of hybrids, and the most useful information on this is obtained from the meiotic pairing in haploids.

The hybrid *A. longissima* × *T. timopheevi* combined the haploid complements of its parent species. The mean bivalent frequency in this hybrid was markedly higher than that expected from a simple summation of the frequencies of the haploids of its parents (table 5). There were also more trivalents. Further, the maximum pairing

possible from combining the haploids would be 4 bivalents whereas the maximum observed in the hybrid was 5 bivalents.

It is clear, therefore, that there was allosyndetic pairing in this hybrid. Without knowledge of the level of structural duplication in the parents, however, all the pairing might have been thought to be autosyndetic. This stresses the importance of utilising information obtained from haploids in the interpretation of the behaviour of interspecific hybrids.

TABLE 5

Mean pairing at metaphase in A. longissima × T. timopheevi F_1 *and the sum of the means of the haploids of the parents*

	univ.	biv.	triv.	s-s	e-e
A. longissima × *T. timopheevi* . .	17·99	1·43	0·05	*0·53*	*0·51*
Haploid *A. longissima*+polyhaploid *T. timopheevi*	20·19	0·38	0·02	*0·66*	*0·47*

7. SUMMARY

1. At first meta-anaphase of meiosis in haploid *Ægilops longissima* ($1x = 7$), polyhaploid *Triticum timopheevi* ($2x = 14$), polyhaploid *Triticum vulgare* ($3x = 21$) and aneupolyhaploid *Triticum vulgare* ($3x+1 = 22$) there were some bivalents, but the majority of chromosomes remained as univalents. In the polyhaploids there were occasional trivalents.

2. There were two main types of univalent associations: side-by-side (s-s) in which the chromosomes lay parallel to each other, and end-to-end (e-e) in which they lay in tandem. End-to-side associations occurred infrequently, and there were rare mixed multiple associations.

3. Members of s-s associations were segmentally homologous but members of e-e's were not. It is suggested that all types of univalent association were due to heterochromatin fusion. Such fusion would maintain the parallel alignment of chromosomes which had paired homologously at prophase, without chiasma-formation, and lead to s-s association. Chance fusions would result in e-e and other associations.

4. Univalents segregated to the poles at random at metaphase in all the plants except polyhaploid *T. timopheevi*. Univalents returned to the equator at random from either pole at late metaphase in polyhaploid *T. vulgare*.

5. Relative to their chromosome numbers *A. longissima* and *T. timopheevi* have about the same amount of structural equivalence within their haploid complements. Both have considerably less than *T. vulgare*. It is suggested that this is due to the development, in the course of evolution, of greater differentiation between the chromosome

sets of the older tetraploid species than between those of the younger hexaploid.

6. The hybrid *A. longissima* × *T. timopheevi* had more chromosome pairing than might have been expected from a simple summation of the pairing in the haploids of the parent species. This demonstrated chromosome homologies between the two species which might otherwise have been unsuspected. The importance is emphasised of using information from haploids in the interpretation of chromosome behaviour in hybrids.

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Plate

Photomicrographs of first metaphase of meiosis in Feulgen stained pollen mother cells of a haploid, two polyhaploids and a triploid hybrid, in the Triticinæ. × 1000.

FIG. 1.—*Ægilops longissima* ($1x = 7$), 0II 7I, an e-e along the equator.

FIG. 2.—*Triticum timopheevi* ($2x = 14$), 0II 14I, 1 e-e (left) and 1 s-s (right).

FIG. 3.—*Triticum vulgare* ($3x = 21$), 1II 19I, a separating s-s at the equator, an e-s and an s-s at the lower pole.

FIG. 4.—*Triticum vulgare* ($3x = 21$), 3II 15I, an e-e at the equator, a multiple association of 3 univalents combining s-s, e-e and e-s at the lower pole.

FIG. 5.—*Æ. longissima* × *T. timopheevi* ($3x = 21$), 3II 15I, 2 e-e's and 1 s-s.

FIG. 6.—*Æ. longissima* × *T. timopheevi* ($3x = 21$), 4II 13I, a multiple association of six, combining s-s, e-e and e-s at the upper pole.

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XY SEGREGATION IN THE CRANE FLY *TIPULA MAXIMA* (DIPTERA : TIPULIDÆ)

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1. INTRODUCTION

In some animals the X and Y chromosomes of the heterogametic sex do not pair at first prophase. Segregation then depends on some mechanism other than chiasma formation. Such heterogametic systems exist in several insect orders but only in the *Hemiptera-Heteroptera* has the mechanism of segregation been explained (Darlington, 1939). Here the X and Y chromosomes are unpaired at first metaphase but regularly congress and divide after the autosomes. The sex chromatids pair momentarily at the second division, co-orientate and then segregate. This behaviour depends upon a special spindle structure and a special centromere cycle.

The present paper is concerned with the analysis of a distinctive type of XY segregation in the male crane fly *Tipula maxima*.

2. MATERIAL AND TECHNIQUE

The testes of crane flies develop in the last (fourth) larval instar. The gonads of pupal and imaginal males consist of maturing spermatids and spermatozoa only. *Tipula maxima* is the largest of British crane flies. The larvæ frequent damp environments, burrowing just below the surface of saturated soil on the margins of woodland streams and pools. Fourth instars may be collected throughout the winter months, but the testes are not in an active state of division until March and April. By this time most of the larvæ are fully grown. The larvæ begin to pupate during April and adults are found from May to August.

The larvæ used in the present study were collected from Ogmore Vale, Glamorgan. I am indebted to T. Parsons of this department for the material and its identification. The larval testes are paired and lie attached to the inner side of the fat body at the junction of the mid and hind gut.

In order to remove the testes the posterior ends of larvæ were cut off under saline (Baker, 1950, p. 182). This results in the fat body floating free. From here the gonads were removed with needles and transferred to 1 : 3 acetic-alcohol prior to squashing in acetic-orcein.

3. OBSERVATIONS

(1) *Mitosis*

The male mitotic complement includes six autosomes and a smaller pair of subequal sex chromosomes ($2n = 6A+XY$). All the chromosomes are metacentric and the mitotic partners show the marked somatic pairing characteristic of dipterans (plate I, fig. 1).

(II) *First meiotic division*

Pre-meiotic resting nuclei contain a large chromocentre which represents the sex chromosomes. Charging of the autosomes begins at the centromere (*cf.* Darlington, 1933). These centric blocks are

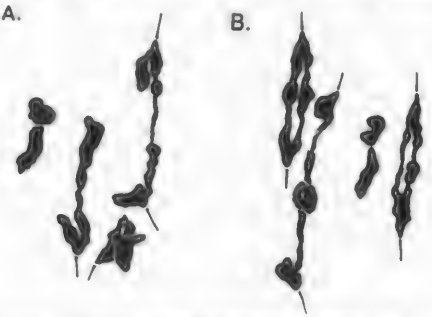

FIG. 1.—Early pre-stretch, subequal sex chromosomes still associated. In (A) two autosomes only are stretched. In (B) all three autosomes are affected. ×2000.

sticky and at zygotene may associate with each other (plate I, fig. 2) or with the persistent sex chromocentre. These regions remain overcharged at pachytene (plate I, fig. 3).

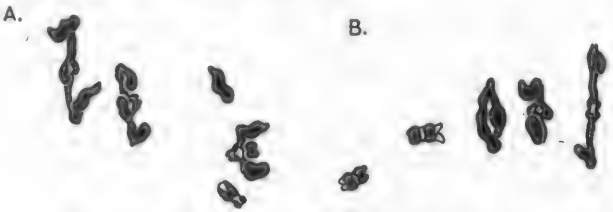

FIG. 2.—(A) Congression and (B) First metaphase. In (A) one sex univalent is still uncoiling; the other is divided and its centromeric region is undercharged (*cf.* plate 2, fig. 3). In (B) both sex univalents have divided and in both the differential heterochromatic segments are undercharged. Note early neo-centromere activity in the undercharged end. ×2000.

The autosomal bivalents generally have two interstitial chiasmata at diplotene and at this stage the sex association can be resolved into two sub-equal chromosomes which are not associated by chiasmata (plate I, fig. 5). Indeed they may exist as univalents which subsequently behave independently (plate I, fig. 4). Following diakinesis there is a pronounced and asynchronous pre-metaphase stretch (*cf.* Hughes-Schrader, 1943; John and Lewis, 1957). This results in the disruption of persistent sex associations, the X and Y then moving to opposite poles (plate I, fig. 8). A pre-metaphase movement has also been observed in living spermatocytes of *Tipula lateralis* (Dietz, 1956).

The sex chromosomes uncoil during pre-stretch and show that they are double (plate II, figs. 3 and 4; fig. 2). They also develop

a regional charge differentiation and can then be seen to consist of four distinct segments :—

(i) A terminal segment, normally charged (like the autosomes).
(ii) An interstitial segment, undercharged, which contains the centromere.
(iii) An interstitial segment normally charged.
(iv) A terminal segment, undercharged.

Terminalisation in the autosomes is incomplete and on recontraction they congress (plate II, fig. 5). The sex univalents generally congress after the autosomes have begun their asynchronous anaphase movement (plate II, figs. 6 and 7). The sex chromosomes divide equationally at first anaphase and are both completely undercharged at this stage. In the separation of the sister sex chromatids one of the ends leads the way to the pole (plate II, figs. 8-11). This frequently results in an arrangement where the sex chromatids form a linear sequence bridging the distance between the telophase groups. During the lag between autosomal anaphase and the division of the sex univalents the spindle increases in length and may become slightly curved.

(iii) *Second meiotic division*

The X and Y chromosomes having divided at the first division do not divide at the second. During second prophase the heterozygous sex chromatids pair. This appears to be controlled by the simultaneous recharging, in the X and Y, of the two segments which decharged precociously at first pro-metaphase. Pairing usually begins at the terminal segments and is consolidated by the association of the two interstitial centric segments (plate III, figs. 2-4). On only two occasions have I found the terminal charged segment of one chromatid in association with the interstitial segment of its partner and vice-versa (plate III, fig. 6). Undercharged regions are not closely associated : they lie parallel and slightly apart.

In this condition the centromeres of the sex pair co-orientate and the association congresses to the edge of the spindle (plate III, fig. 5). Segregation of the X and Y seems to depend on two forces :—

(i) Active repulsion between the centric segments (plate III, figs. 7 and 8).
(ii) Active mobility in the undercharged ends (plate III, figs. 5 and 9-12).

4. DISCUSSION

In all other XY tipulid species so far studied the sex chromosomes agree with those of *T. maxima* in that they do not pair at prophase of meiosis (Bauer, 1931 ; Wolf, 1941 ; Dietz, 1956, and Bauer, 1956, quoted in Dietz). In these forms, however, the X and Y chromosomes

segregate at first anaphase and then divide equationally at second meiosis. White (1949) compared this type of segregation with the distance pairing of neuropteran sex chromosomes (Naville and de Beaumont, 1933, 1936) and on this basis suggested that the tipulids evolved from neuropteroid ancestors. There is nothing to support this suggestion ; the sex univalents move onto the equator at first anaphase and only after this do they move undivided to opposite poles. How this regular segregation is controlled has never been discussed.

At the onset of meiosis in *T. maxima* both sex chromosomes are positively charged and are associated non-specifically. This is probably due to the characteristic stickiness of heterochromatin (Darlington, 1947 ; Pätau, 1948 ; Vaarama, 1954). The lapse of this association during first prophase similarly can be correlated with a progressive reduction in charge. This, together with the pre-stretch, results in the X and Y chromosomes separating as two univalents.

In *Cimex* (Darlington, 1939) and *Luzula* (Thomas, 1950) centromere precocity has been shown to follow overcharging at first prophase. The same correlation is found in *T. maxima*. At pre-stretch the univalents move towards one pole ; presumably therefore their centromeres must auto-orientate at attachment to the spindle. But the univalents invariably return to the equator (*cf.* Dietz, 1956). Probably, therefore, the univalent centromeres re-orientate during pre-stretch. In heterotypi cchromosomes the metaphase centromere, although undercharged, cannot be identified. This could be due to the centromere being screened by the meiotic spiral (Östergren, 1951). In *T. maxima* the sex univalents uncoil during pre-stretch. This could lead to the disappearance of the centric screen so that the centromere then becomes functionally bilateral, accounting for the return of the univalents from the poles to the equator.

The centromeres are not the only kinetic centres active during meiosis. Secondary centres occur in one of the end segments of both the X and Y. The kinetic potentiality of these active ends results in their poleward flexion so that at both meiotic divisions these ends lead the way to the poles. This behaviour of the terminal and secondary centres identifies them as neo-centromeres (Vilkomerson, 1950 ; Walters, 1952). The nucleic acid cycle of the sex chromosomes of *T. maxima* is anomalous. Secondary centric activity in this species may therefore be explained in terms of Darlington's (1950) diffusion theory.

Two series of observations support the suggestion that the activity of the sex neo-centromeres is adaptive :—

(i) Undercharged segments lead to a failure of separation (Darlington and La Cour, 1940 ; Koller, 1946). The sex neo-centromeres develop only in the undercharged terminal segments.

Plate I

FIG. 1.—Mitotic pro-metaphase ($2n = 8$) ; strong somatic pairing. Subequal sex chromosomes at seven o'clock. ×2000.

FIG. 2.—Zygotene. Three autosomal centric blocks fused in a heterochromatic sticky association. Persistent sex chromocentre at seven o'clock. ×1400.

FIG. 3.—Pachytene. Autosomes with precharged proximal blocks. Sex chromocentre at six o'clock. ×1400.

FIG. 4.—Diplotene. Autosomes chiasmate. Sex association disrupted. ×1400.

FIG. 5.—Diplotene. Sex association analysable into two subequal chromosomes but persistent. ×1400.

FIG. 6.—Diakinesis. Sex association clearly non-chiasmate. ×1400.

FIG. 7.—Early pre-stretch. Sex association disrupting. Note asynchrony of autosomal movement. ×1400.

FIG. 8.—Mid pre-stretch. Autosomal stretching markedly asynchronous. Sex univalents widely separate. ×1400.

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Plate II

FIG. 1.—Early recontraction. Note asynchrony of behaviour. × 1400.

FIG. 2.—Mid recontraction. Sex univalents still maximally separated and uncoiling. × 1400.

FIG. 3.—Late recontraction. × 1400. Compare with fig. 2A.

FIG. 4.—Early congression. Note differential charging of the divided univalents. × 1400.

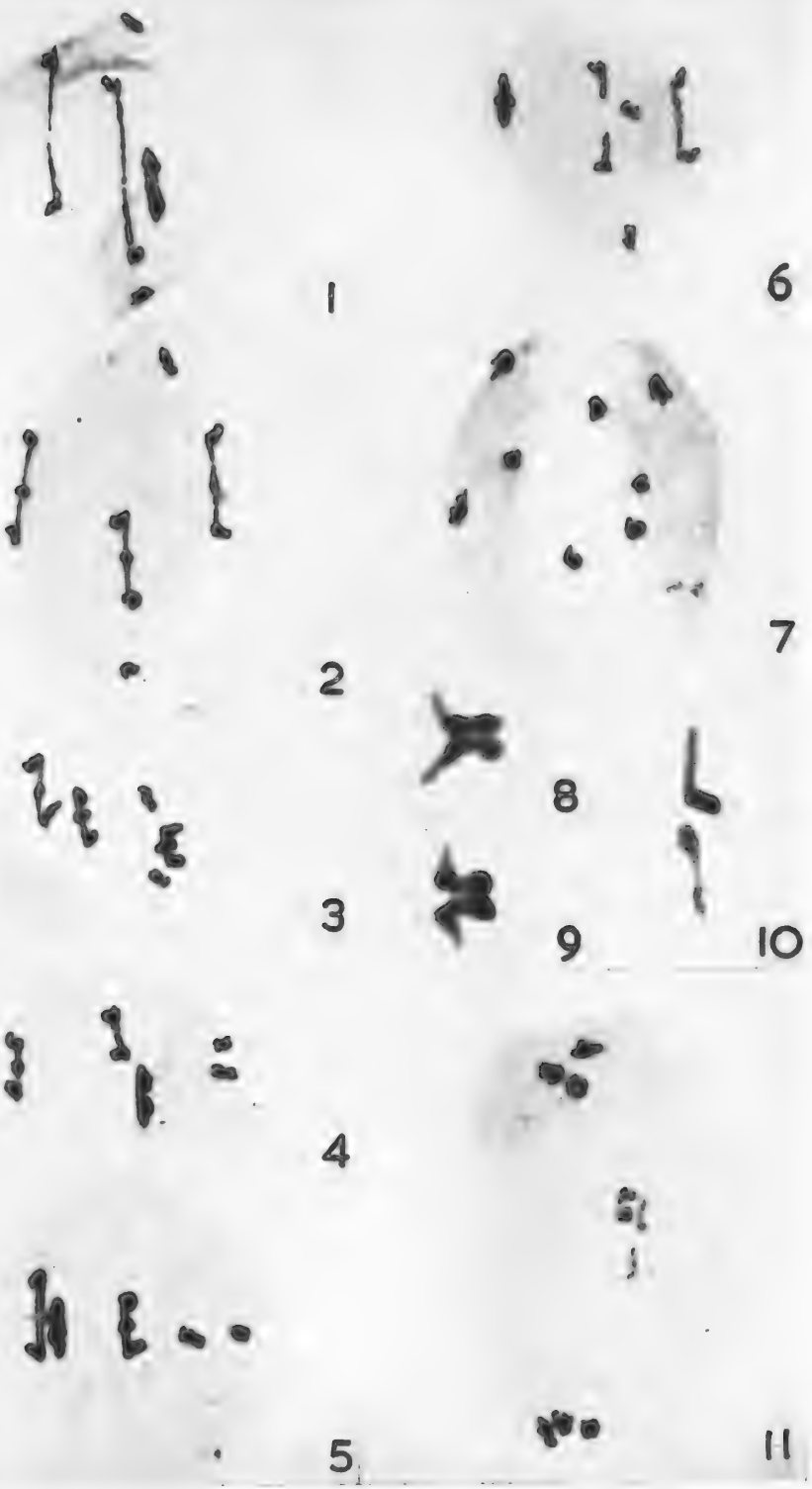
FIG. 5.—Metaphase I. Sex univalents lying on the edge of the spindle. Terminalisation incomplete. × 1400.

FIG. 6.—Early anaphase I. Note asynchrony of separation. × 1400.

FIG. 7.—Mid anaphase I. Divided univalents aligned on spindle equator. × 1400.

FIGS. 8, 9 and 10.—Progressive stages in the separation of daughter sex chromatids at anaphase I as the result of neo-centric activity in the undercharged chromatid ends. × 4000.

FIG. 11.—Late anaphase I. Sex chromatids separated, undercharged and lagging. Note increased spindle length. × 1400.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(ii) The centromeres of univalents are characteristically weak (Darlington, 1939, 1947). The sex neo-centromeres seem to be strong since at both divisions they may become active before the autosomal centromeres (plate III, fig. 5).

Neo-centric activity also occurs in one autosome which is not heterochromatic. This activity is variable ; it is most pronounced at the second division (plate III, fig. 5).

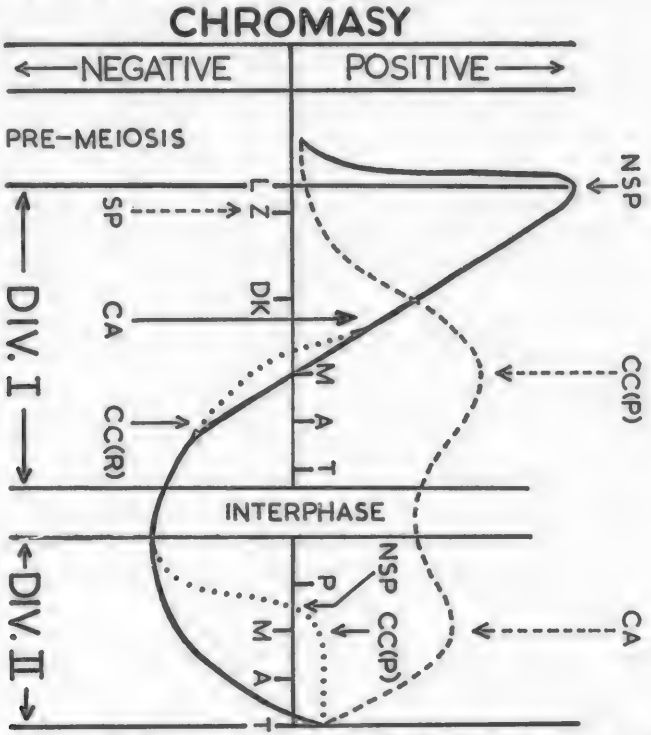

FIG. 3.—Diagram to illustrate the meiotic allocycly of the sex chromosomes of *Tipula maxima* and its relation to autosomal charging. The broken line and broken arrow arms refer to the euchromatic autosomes (- - -). The solid line and solid arrow arms refer to the heterochromatic sex chromosomes (——). The dotted line refers to the two heterochromatic segments with precocious allocycly (. . .).
SP = Specific pairing : NSP = Non-specific pairing.
CC(P) = Centromeres co-orientated (products of pairing).
CC(R) = Centromeres co-orientated (products of reproduction).
CA = Centromeres auto-orientated.

Both sex chromosomes consist of two kinds of heterochromatin differing from each other, as well as from euchromatin, in their charge cycle. These two kinds alternate with one another so that the X and Y each consist of four segments. One end and a centric segment have the same cycle ; the segment between these two and the other end having a different cycle (fig. 3). At the second division the two

former segments recharge precociously and this seems to regulate the association of the heterozygous sex chromatids. This in turn determines the co-orientation of the sex centromeres on the second division spindle.

These two segments with a precocious charging may represent adaptively modified pairing segments. The only other cases where two distinct heterochromatic cycles have been described within one chromosome are those which relate to the pairing and differential segments of certain mammalian sex chromosomes (Koller, 1938; 1946; Pontecorvo, 1943, 1944; Sharman and Barber, 1952). In a limited sense, therefore, one can still speak of " pairing " segments in *T. maxima* although these segments enjoy no gene exchange.

5. SUMMARY

1. In *T. maxima* the X and Y chromosomes do not pair at zygotene but are associated non-specifically at the onset of meiosis by the stickiness of heterochromatin.

2. Relaxation of the XY association during first prophase is correlated with a progressive reduction in charge. This, together with the pre-metaphase stretch, results in their separating into two univalents.

3. The sex univalents divide at first anaphase. At second metaphase the X and Y pair with one another and their centromeres co-orientate. This association is regulated by the precocious and localised recharging of two segments.

4. The equational first division of the sex chromosomes has entailed the development of a special mechanism of segregation at second anaphase. This is related both to the reversed centromere cycles of the X and Y and the development of neo-centric activity in the undercharged terminal segments.

I wish to thank Professor C. D. Darlington for criticism and advice in the preparation of this paper.

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Plate III

FIG. 1.—Early prophase II. Heterozygous and subequal sex chromatids unpaired, undercharged and despiralising. ×4000.

FIGS. 2, 3 and 4.—Progressive stages in the pairing of the heterozygous sex chromatids during second prophase. ×4000.

FIG. 5.—Metaphase II. Note marked neo-centric activity in the terminal segment of one of the autosomal chromatids and in the undercharged end segments of both sex chromatids. ×1400.

FIG. 6.—Alternative pairing of sex chromatids by the reverse association of pairing blocks. ×4000.

FIGS. 7, 8, 9 and 10.—Progressive stages in the separation of the heterozygous sex chromatids at second meta-anaphase. ×4000.

FIG. 11.—Anaphase II. Slight lagging of sex chromatids. ×1400.

FIG. 12.—Telophase II. Note terminal neo-centric activity in the sex chromatids and in one of the autosomes. ×1400.

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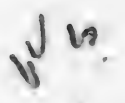

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PARTIAL MANIFESTATION OF A GENE IN COMPLETE THREE AND HIGHER POINT BACKCROSS DATA

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1. INTRODUCTION

IMPERFECT penetrance of a gene in a linkage experiment leads to considerable disturbance in the data, frequently of far greater magnitude than that caused by differential viability, which usually needs very large numbers to reveal its presence. The problem of estimating the degree of misclassification was considered by Smith (1937), but of far greater importance is the question of finding accurate estimates of the recombination values. Two-point backcross data have been treated adequately by Bailey (1950) and Sánchez-Monge (1952) on theoretical grounds. Fisher (1953*a*) used Bailey's method to estimate the recombination fraction between polydactyly (*Py*), which is imperfectly penetrant, and leaden (*ln*) in the thirteenth chromosome of the house mouse, but he modified Bailey's estimates of the sampling variances.

In this paper it is proposed to extend the methods developed for two-point data to complete three and higher point backcross data. By complete we shall mean a backcross in which data have been collected for all possible heterozygous parents. In the case of a three-point test there are four possible heterozygotes, and in a four-point there are eight.

Furthermore, this paper considers the simplest possible situation, which is that of the partial manifestation of one gene unaffected by the partial manifestation or differential viability of any other gene. Differential viability in a linkage experiment is often largely catered for without further calculation if the experiment is adequately balanced (*i.e.* there are approximately an equal number of zygotes in each of the possible mating types).

Before considering three and higher point data, the method proposed by Fisher (1953*a*) for two-point data will be reviewed, as the treatment of higher point data merely extends this.

2. TWO-POINT DATA

Consider a backcross $\frac{AB}{ab} = \frac{ab}{ab}$ in which a is misclassified such that $a \rightarrow A$, the wild type allele. Let λ be the misclassification and p the recombination fraction between A and B such that $p+q = 1$.

Observations and expectations may now be drawn up :—

	AB	*ab*	*Ab*	*aB*	Total
Observed	*a*	*b*	*c*	*d*	*n*
Expected	$\frac{1}{2}(q+\lambda p)$	$\frac{1}{2}q(1-\lambda)$	$\frac{1}{2}(p+\lambda q)$	$\frac{1}{2}p(1-\lambda)$	1

To find the estimates $\hat{p}$ and $\hat{\lambda}$ we may proceed as follows :—

$$p(1-\lambda) = \frac{d}{a+d} = \alpha = \text{proportion of } a \text{ in } B \text{ phenotypes}$$

$$q(1-\lambda) = \frac{b}{b+c} = \beta = \text{proportion of } a \text{ in } b \text{ phenotypes.}$$

Adding $$1-\hat{\lambda} = \alpha+\beta \quad . \quad . \quad . \quad . \quad . \quad . \quad (2.1)$$

and $$\hat{p} = \frac{\alpha}{\alpha+\beta} \quad . \quad . \quad . \quad . \quad . \quad . \quad (2.2)$$

these forms being equivalent to those given by Bailey (1950).

The quantities α and β are respectively proportions observed of the manifesting class out of the number $a+d$ of B phenotypes, and the number $b+c$ of b phenotypes. Thus α and β can be regarded as determined conditional on $a+d$ and $b+c$ respectively, and so have binomial variances.

$$V(\alpha) = \frac{\alpha(1-\alpha)}{a+d}, \; V(\beta) = \frac{\beta(1-\beta)}{b+c} \quad . \quad . \quad (2.3)$$

The variances $V(p)$ and $V(\lambda)$ are calculated as follows.

From equation (2.1)

$$V(\lambda) = V(1-\lambda) = V(\alpha+\beta) \quad . \quad . \quad . \quad (2.4)$$

Since α and β are confined to two non-overlapping totals, the covariance term in $V(\alpha+\beta)$ will be zero. Therefore

$$V(\lambda) = V(\alpha)+V(\beta) \quad . \quad . \quad . \quad . \quad (2.5)$$

We may find $V(p)$ using the relationship :—

$$V(p) = \left(\frac{\partial p}{\partial \alpha}\right)^2 V(\alpha) + \left(\frac{\partial p}{\partial \beta}\right)^2 V(\beta) + 2\,\frac{\partial p}{\partial \alpha}\,.\,\frac{\partial p}{\partial \beta}\,V(\alpha\beta)$$

(Fisher, 1953*b*)

As α and β are independent, the covariance term in this expression is zero and we obtain :—

$$V(p) = \frac{\beta^2 V(\alpha)+\alpha^2 V(\beta)}{(\alpha+\beta)^4} \quad . \quad . \quad . \quad (2.6)$$

The variances $V(p)$ and $V(\lambda)$ are calculated in terms of α, β, $V(\alpha)$ and $V(\beta)$ which are in turn dependent upon the observed numbers a, b, c, d. Hence $V(p)$ and $V(\lambda)$ are calculated from the information realised. In the formulæ (2.7, 2.8) given by Bailey (1950) $V(p)$ and $V(\lambda)$ are based upon the information expected (for a given total n).

Bailey gives :—

$$V(\lambda) = \frac{2(1-\lambda)}{n}\,\{2pq+\lambda(1-2pq)\}\,. \quad . \quad . \quad (2.7)$$

$$V(p) = \frac{2pq}{n}\left\{\frac{1}{1-\lambda}-2pq\right\} \quad . \quad . \quad . \quad . \quad (2.8)$$

which are equivalent to Fisher's formulæ (2.5, 2.6) when

$$a+d = b+c = \tfrac{1}{2}n \quad . \quad . \quad . \quad . \quad (2.9)$$

This relation (2.9) is derived from the expectations of the four phenotypes *AB*, *ab*, *Ab* and *aB*.

Hence $V(p)$ and $V(\lambda)$ calculated as information realised will almost certainly be different to the information expected and in fact are only equivalent when the condition (2.9) holds. Fisher (1953*a*) gives a case for the linkage between *py* which is imperfectly penetrant and *ln* for males in coupling where this condition holds. In this set of data the goodness of fit χ^2_1 is zero.

TABLE 1

Expectations, admitting misclassification of gene a

	E		*F*	
	ABC	*abc*	*aBC*	*Abc*
tricoupling . .	$\frac{1}{2}(p+\lambda q)a$	$\frac{1}{2}(p-\lambda p)a$	$\frac{1}{2}(q-\lambda q)a$	$\frac{1}{2}(q+\lambda p)a$
a out . . .	$\frac{1}{2}(q+\lambda p)b$	$\frac{1}{2}(q-\lambda q)b$	$\frac{1}{2}(p-\lambda p)b$	$\frac{1}{2}(p+\lambda q)b$
c out . . .	$\frac{1}{2}(r+\lambda s)c$	$\frac{1}{2}(r-\lambda r)c$	$\frac{1}{2}(s-\lambda s)c$	$\frac{1}{2}(s+\lambda r)c$
b out . . .	$\frac{1}{2}(s+\lambda r)d$	$\frac{1}{2}(s-\lambda s)d$	$\frac{1}{2}(r-\lambda r)d$	$\frac{1}{2}(r+\lambda s)d$
	G		*H*	
	*ab*C	ABc	*a*Bc	A*b*C
tricoupling . .	$\frac{1}{2}(r-\lambda r)a$	$\frac{1}{2}(r+\lambda s)a$	$\frac{1}{2}(s-\lambda s)a$	$\frac{1}{2}(s+\lambda r)a$
a out . . .	$\frac{1}{2}(s-\lambda s)b$	$\frac{1}{2}(s+\lambda r)b$	$\frac{1}{2}(r-\lambda r)b$	$\frac{1}{2}(r+\lambda s)b$
c out . . .	$\frac{1}{2}(p-\lambda p)c$	$\frac{1}{2}(p+\lambda q)c$	$\frac{1}{2}(q-\lambda q)c$	$\frac{1}{2}(q+\lambda p)c$
b out . . .	$\frac{1}{2}(q-\lambda q)d$	$\frac{1}{2}(q+\lambda p)d$	$\frac{1}{2}(p-\lambda p)d$	$\frac{1}{2}(p+\lambda q)d$

3. THREE-POINT DATA

A three-point backcross will now be considered in which the four possible triply heterozygous parents are represented. Let the two segments between the three linked genes *a*, *b*, *c* be (1) and (2). Let *p*, *q*, *r*, *s* be the frequencies of the modes of gamete formation (0), (1), (2), (12) and *a*, *b*, *c*, *d* be proportional to the number of zygotes in each mating type such that

$$p+q+r+s = 1$$
$$a+b+c+d = 1$$

and let λ = misclassification of gene *a* such that $a \rightarrow A$.

Let *E*, *F*, *G* and *H* represent the four pairs of complementary genotypes :—

E *ABC*, *abc*
F *aBC*, *Abc*
G *abC*, *ABc*
H *aBc*, *AbC*

For each complementary pair, admitting misclassification of gene *a*, expectations may be drawn up (table 1).

These expectations may be re-arranged as a 4×4 Latin Square of the form :—

$$\begin{matrix} E & F & G & H \\ F & E & H & G \\ G & H & E & F \\ H & G & F & E \end{matrix}$$

in which the columns correspond to the four modes of gamete formation, the rows correspond to the four mating types, and the letters to the four pairs of complementary genotypes. The re-arranged expectations, after adding the members of each complementary pair, are tabulated below (table 2).

TABLE 2

Expectations arranged in a Latin Square

	Parental	*a* out	*c* out	*b* out	
tricoupling . .	$2p+\lambda(q-p)$	$2q+\lambda(p-q)$	$2r+\lambda(s-r)$	$2s+\lambda(r-s)$	$\times \frac{a}{2}$
a out . . .	,,	,,	,,	,,	$\times \frac{b}{2}$
c out . . .	,,	,,	,,	,,	$\times \frac{c}{2}$
b out . . .	,,	,,	,,	,,	$\times \frac{d}{2}$

The expected column totals in table 2 may be equated to the observed column totals which we can represent by P, Q, R and S respectively. We obtain (remembering that $a+b+c+d=1$) four equations :—

$$p+\tfrac{1}{2}\lambda(q-p) = P \quad . \quad . \quad . \quad . \quad (3.1)$$
$$q+\tfrac{1}{2}\lambda(p-q) = Q \quad . \quad . \quad . \quad . \quad (3.2)$$
$$r+\tfrac{1}{2}\lambda(s-r) = R \quad . \quad . \quad . \quad . \quad (3.3)$$
$$s+\tfrac{1}{2}\lambda(r-s) = S \quad . \quad . \quad . \quad . \quad (3.4)$$

Adding (3.1) and (3.2)

$$p+q = P+Q$$

and substituting in (3.1)

$$\left.\begin{aligned} p &= \frac{P-\frac{1}{2}\lambda(P+Q)}{1-\lambda} \\ \text{and} \quad q &= \frac{Q-\frac{1}{2}\lambda(P+Q)}{1-\lambda} \end{aligned}\right\} \quad . \quad . \quad . \quad (3.5)$$

Similarly

$$\left.\begin{aligned} r &= \frac{R-\frac{1}{2}\lambda(R+S)}{1-\lambda} \\ \text{and} \quad s &= \frac{S-\frac{1}{2}\lambda(R+S)}{1-\lambda} \end{aligned}\right\}$$

From the calculated values of p, q, r and s (3.5), which are the four modes of gamete formation, the three possible recombination values $(q+r)$, $(r+s)$, and $(q+s)$ may be calculated by simple addition since $p+q+r+s=1$.

The misclassification, λ, may be calculated by a method analogous to that employed for two-point data.

Let
$$\begin{aligned} ap+bq+cr+ds &= \mathcal{J} \\ aq+bp+cs+dr &= K \\ ar+bs+cp+dq &= L \\ as+br+cq+dp &= M \end{aligned}$$

From table 1

$$a \text{ in } BC = \frac{\text{total observed } aBC}{\text{total observed } (aBC+ABC)} = \frac{K(1-\lambda)}{\mathcal{J}+K} = \alpha$$

$$a \text{ in } bc = \frac{abc}{abc+Abc} = \frac{\mathcal{J}(1-\lambda)}{\mathcal{J}+K} = \beta$$

$$a \text{ in } bC = \frac{abC}{abC+AbC} = \frac{L(1-\lambda)}{L+M} = \gamma$$

$$a \text{ in } Bc = \frac{aBc}{aBc+ABc} = \frac{M(1-\lambda)}{L+M} = \delta$$

Adding

$$1-\lambda = \tfrac{1}{2}(\alpha+\beta+\gamma+\delta) \quad . \quad . \quad . \quad . \quad (3.6)$$

and also

$$V(1-\lambda) = \tfrac{1}{4}[V(\alpha)+V(\beta)+V(\gamma)+V(\delta)] \quad . \quad . \quad (3.7)$$

where $V(\alpha)$, $V(\beta)$, $V(\gamma)$ and $V(\delta)$ are calculated as for the two-point situation.

The variances of the recombination fractions $(r+s)$, $(q+r)$, and $(q+s)$ are most easily found by considering them separately as two-point data.

The recombination fraction $(r+s)$ measures recombination in segment bc and is therefore independent of the partial manifestation of gene a. The variance is therefore found in the normal way :—

$$V(r+s) = \frac{(r+s)(1-r+s)}{n}$$

Now $(q+s)$ measures recombination in segment ab. In table 1, by ignoring gene c we can calculate α_1 and β_1 the proportion of a in B and a in b respectively. Thus we find :—

$$V(q+s) = \frac{\beta^2{}_1 V(\alpha_1)+\alpha^2{}_1 V(\beta_1)}{(\alpha_1+\beta_1)^4} \quad . \quad . \quad . \quad (3.9)$$

And similarly, by ignoring gene b, $V(q+r)$ may be found.

It must finally be noted that if gene b or c were misclassified instead of a, two similar but not identical sets of four expressions for p, q, r and s would be obtained.

4. FOUR-POINT DATA AND MORE

The method for four-point data is merely an extension of that for three-point data. Let the three segments between the four linked genes a, b, c, d be (1), (2) and (3). Let the frequencies of the eight modes of gamete formation (0), (1), (2), (3), (12), (13), (23) and (123) be p, q, r, s, t, u, v and w respectively such that

$$p+q+r+s+t+u+v+w = 1$$

By arranging the data from the eight possible four-fold heterozygotes in an 8×8 Latin Square eight equations can be derived. The following set of eight equations is derived for misclassification of gene d.

$$\begin{aligned}
p+\tfrac{1}{2}\lambda(s-p) &= P\\
q+\tfrac{1}{2}\lambda(u-q) &= Q\\
r+\tfrac{1}{2}\lambda(v-r) &= R\\
s+\tfrac{1}{2}\lambda(p-s) &= S\\
t+\tfrac{1}{2}\lambda(w-t) &= T\\
u+\tfrac{1}{2}\lambda(q-u) &= U\\
v+\tfrac{1}{2}\lambda(r-v) &= V\\
w+\tfrac{1}{2}\lambda(t-w) &= W
\end{aligned}$$

where P, Q, R, S, T, U, V, W are the observed column totals of the 8×8 Latin Square.

From the above set of eight equations, the frequencies of the eight modes of gamete formation can be calculated. This method will be equally applicable to higher point data.

5. SUMMARY

A method for allowing for partial manifestation of a gene in complete three and higher point backcross data is proposed. This is needed in the treatment of four-point data such as that being collected in this Department for the chromosome XIII markers *py*, *ln*, *fz* (fuzzy) and *Sp* (Splotch) of the house mouse by Professor Sir Ronald Fisher.

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A BALANCED THREE-POINT EXPERIMENT FOR LINKAGE GROUP V OF THE HOUSE MOUSE

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1. INTRODUCTION

Some fourteen linkage groups are now available in the house mouse, *Mus musculus*. In some of these, as many as seven or eight factors have been located. In many groups, the order of the various loci has been reliably ascertained, and some of the recombination fractions for adjacent loci have been accurately established. However, the chromosomes have not yet been mapped in a thorough and detailed manner: there is much to be done before it is possible to specify the frequencies of the modes of gamete formation of multiply-heterozygous animals—that is, before chromosome maps can be used to predict the outcome of experimental programmes involving many linked loci, such as will no doubt be essential to the fields of applied genetics of the future.

The obstacles retarding progress towards this end appear to be two-fold. Firstly, knowledge about the phenomenon of interference is still elementary. Some preliminary experimental work has been reported, and much theoretical work has been done—in fact, theory is greatly in advance of observation. Little progress appears possible until further data are at hand.

Secondly, experimental material, in its turn, has often been neither sufficiently comprehensive nor sufficiently accurate to allow quantitative rather than merely qualitative conclusions concerning interference to be drawn from it. Such inaccuracy is usually found to be due to one or all of the following causes: poor design, inadequate practical execution, and imperfect analysis of results.

The experiment now to be reported is not free from imperfections, nor does it present any basically new techniques or ideas. It is an attempt to make practical use of relevant theory in design, construction and analysis, and in so doing to make a small but reliable contribution to the study of interference.

2. DESIGN: SOME HISTORICAL NOTES

The need for homogeneity in linkage data was recognised some forty years ago. Bridges (1915) and Muller (1916) devised a very competent method of eliminating differential viability, a method which uses what is now termed "balance". Further, Bridges and Morgan (1923) listed a number of factors capable of causing heterogeneity in *Drosophila* data: fluctuation of recombination value with age, with the chromosome involved and even with different sections of the same chromosome,

response to temperature and other environmental agents, and " a large number of mutant conditions which decrease, or, more rarely, increase the amount of crossing-over ".

It is surprising, therefore, to find that Bridges' and Muller's concept of balance, which can be used to overcome one of these difficulties, was seldom used for two-point work, very rarely for three-point, and not at all for work involving more than three factors.

Bridges in his 1915 paper states : " In obtaining data upon any linkage case it is best to have half the data in the form of ' coupling ' and half as ' repulsion ' experiments. . . . We may offset . . . disturbance by balancing the viability of a certain class against itself." He then sets out two tables of data balanced in this way. It is quite clear that this method was intended for experiments involving more than two loci, for Bridges continues " To balance the data of an experiment involving three loci requires four sets of data, instead of two, as in the case of two loci," and a further treatise by Bridges and Morgan (1916) states : " For three pairs of genes there are eight classes, and in order that each of them may appear as a non-cross-over, as each single cross-over, and as the double cross-over, four experiments must be made." In fact a three-point experiment using all four kinds of heterozygote is tabled here for vermilion, sable and bar : about 7500 flies were bred in all. Another three-point, with imperfect balance (very unequal numbers of progeny were raised from the four heterozygotes) is reported by Bridges and Morgan (1919), using black, purple and vestigial : about 5000 flies were classified. A further three-point experiment, on a very large scale, is described by the same authors (1923), using dichaete, ski-III and spineless ; more than 15,000 flies were bred.

It is in the 1923 paper that a reason for the paucity of good multi-point data becomes apparent. It is there stated that not only does " the combination of the data from these four crosses give a neutralisation of the effect of inviability," but " this method has the further great advantage that since the sources and relations of the chromosomes are varied, cross-over variations are also neutralised or revealed ".

The mistaken idea that all possible causes of heterogeneity are cancelled out by balance probably led indirectly to the abandonment of this method : for a workman who expects too much of his tool discards it when it does not fulfil his expectations. Certainly, a scrutiny of the published *Drosophila* three-points reveals a degree of heterogeneity which renders impossible an accurate interpretation of the operation of interference. In some cases there is heterogeneity of output between heterozygous types, in others between matings of the same type ; and the source of heterogeneity is sometimes found to be due to (possibly real) fluctuation in recombination values, and sometimes cannot be traced at all, due to inadequate presentation of the data.

Perhaps the most surprising historical feaure is that the balanced design has not been used extensively elsewhere. In the house mouse, in particular, it should be expected to be of great practical value ; for neither of the main causes of fluctuation in the recombination value in *Drosophila*, namely age and temperature variation, have been established sufficiently strongly in mice to arouse the suspicion that " balanced " experiments may be heterogeneous without standardised temperature and age factors. Moreover, it should be comparatively easy to standardise these factors, if it should become necessary. Certainly the first balanced three-point in mice, reported by Wright (M. E. Wallace) in 1947, is remarkably homogeneous.

One may surmise that a further reason why balance was not generally envisaged in the planning of multi-point experiments is that the conditions for perfect balance were not generally realised—and were not understood even by Bridges. Nowhere does he mention the mathematical process whereby " cancellation " can be effected. This situation is strongly indicated by his insistence, even for two-point work, on equal numbers of classified progeny from each kind of heterozygote, an idea later improved by Fisher (1935-53). Muller (1916)—whose description of the same

method of balancing data is apparently independent, since he gives no reference to Bridges' earlier paper—does introduce some mathematical considerations. His main contribution to the understanding of the problem is his proposal that the best estimate of the recombination value is not obtained from the sum of the recombinants from both coupling and repulsion, but is derived from the square root of the following : the products of non-recombinants of both phases divided by the products of recombinants. His treatment falls short of Fisher's in that he does not consider the members of a complementary pair of genotypes as one unit and uses only half of the data. Neither does he give a method of estimating the standard error. No one among the early Drosophilists seems to have applied Muller's treatment to actual data.

It is apparent from a glance at the early *Drosophila* work that the development of statistical methods had not reached a stage when it could be of much use in this problem ; and in fact, it is with admiration that one realises the acuity of judgment with which the enormous volume of work done by these pioneers was compiled and edited by them. It was not until the development of the Method of Maximum Likelihood by Fisher (1912 and 1921, 1925-1954), that the statistical foundations for modern linkage work were laid. As an outcome of this, papers by Hutchinson (1929) and Immer (1934) are noteworthy. Fisher (1935-1953), in giving a thorough treatment of two-point work, shows how the totals from each phase of linkage may be adjusted during the course of an experiment so as to secure the greatest precision for a given number of organisms bred. These adjustments result in equality of *zygotic outputs* from each phase ; this condition (rather than equality of the totals of classified progeny) allows an accurate estimation of the recombination fraction—that is, unbiassed by viability disturbance—to be made by the simple sum of the recombinants from both phases. In addition, Fisher gives an efficient formula for the estimation of the recombination fraction when the zygotic totals are unequal ; this is similar to Muller's, but uses the whole data.

The concept of balance as proposed by Fisher can be extended to multi-point data. There is no direct way such as that available for two points, by which one can calculate the numbers required to be bred from each type of heterozygote in three-point work so that the totals of zygotes produced by each are the same. Nor is there a simple formula for the recombination fraction when they are different. If, on the other hand, viability disturbance is not extreme and approximately equal numbers are bred from each type of heterozygote, a good estimate can be made by the addition method. In designing a test for linkage of six factors and sex, Fisher and Mather (1936*b*) listed, for up to eight factors, the minimum number of types of heterozygotes required in order to obtain both coupling and repulsion data for all pairs of factors. These ideas, which were later used by him (Fisher, 1948) in the design of Wright's three-point test and made generally available (Fisher, 1954) are inherent in Bridges' concept of balance.

The analysis of Wright's experiment, which tested the linkage of $wavy_2$, $shaker_2$ and sex, was made under his direction, and included methods of detecting viability disturbance, should any be present. No significant disturbance was found, hence efficient estimates of the recombination values could be made by the addition method.

A more sensitive method of disclosing disturbance and for correcting for it was then produced by Fisher (1949*a*) : this did not disclose significant disturbance in these data, but the method is now available for use in future work, and has been applied to the three-point data from the experiment involving chromosome V now to be described.

A method of eliminating viability disturbance closely related to that of Fisher has been devised by A. R. G. Owen (unpublished) ; and its application to *Drosophila* data is demonstrated by P. A. Parsons (1957).

Parsons' paper describes the first balanced experiment in *Drosophila* in which the common environmental sources of heterogeneity inherent in this species, which

are not due to viability disturbance, are eliminated by the design of the experiment. With viability disturbance also eliminated during the analysis of the results, he is thus able to draw reliable conclusions both about linkage relations and about certain unusual fluctuations within them.

Besides Wright's experiment in the house mouse there has been one other series of balanced three-points with sex as one of the markers, that of Carter and Phillips (1953). This paper, and some cytological work by Carter, Lyon and Phillips (1954) and Slizynski (1954) cast doubts on the reality of the apparent linkage of $wavy_2$ and $shaker_2$ with sex, demonstrated by Wright. An appraisal of the present evidence on this subject will be published shortly elsewhere : certain discrepancies in Carter and Phillips' data will be discussed, and new evidence in favour of the linkage will be given.

The present experiment with mice was completed in 1952 and received preliminary publication as part of a thesis (Wallace, 1954*a*). As the first balanced three-point in this species which certainly concerns linked genes, it may thus be used unconditionally as a contribution towards a knowledge of interference. A balanced four-point experiment involving the same chromosome has been completed by Owen and a preliminary report (1953*a*) given on the results. A further balanced four-point experiment, involving linkage group XIII, is being carried out by Fisher. This is particularly interesting since it involves polydactyly (*py*), a factor which shows imperfect manifestation. The only balanced multiple linkage experiment which has, apparently, been carried out with a species other than *Drosophila* and mice, is that of Anderson (1921) in maize ; and here the interference relations are somewhat obscured by misclassification ; this the author did much to eliminate from the data but did not consider in the analysis. A balanced five-point, involving linkage group V and using the well-spaced markers Ragged (*Ra*), agouti (*A*), wellhaarig (*we*), fidget (*fi*) and Danforth's short tail (*Sd*), is under construction by the present writer.

3. CONSTRUCTION

The loci concerned in the three-point experiment now to be described are agouti, fidget and Danforth's short tail. A brief resumé of the history of linkage group V will relate these loci to the others in the group.

The loci marked by kreisler (*kr*), agouti (*A*), undulated (*un*), wellhaarig (*we*), and pallid (*pa*), were found by various workers during the interval 1935-49 to be on the same chromosome. Fisher (1949*b*), reporting on their order, gave approximate map distances as :

kr 3 *a* 5 *un* 10 *we* 3 *pa*

The year 1950 saw several developments. Carter and Grüneberg reported a linkage of about 33·5 per cent. between *fi* and *A*, and the present writer a linkage of about 25 per cent. between *Sd* and *fi*. Earlier unpublished evidence collected at the Jackson Memorial Laboratory, Bar Harbor, and at Cambridge by myself, had shown indications of a loose linkage—about 40 per cent., between *A* and *Sd*. Thus the order

A *fi* *Sd*

was strongly indicated.

The relation between this series, and the one known earlier, namely *kr—a—un—we—pa*, was settled by Borger in the same year

(1950), using *A*, *pa* and *Sd*; he gave the order *kr—A—un—we—pa—fi—Sd*. Complementary evidence that *fi* is beyond the segment *A—pa* and nearest to *pa* was furnished by Carter the following year (1951).

The shorter intervals in the segment *kr—pa* have been carefully mapped: Fisher (1949*b*) has given very full data on the interval *A—un*; and Fisher and Landauer (1953) and Owen (1953*a*) have given interim reports on the four-point involving *A*, *un*, *we*, and *pa*, already mentioned. It seemed profitable to map accurately the unexplored *fi—Sd* region, and to investigate interference relations over a wider interval than that examined by Owen: accordingly agouti was chosen as the third locus.

A further reason for the choice of these loci was their suitability for demonstrating the advantage of attaining balanced backcross data in eliminating viability disturbance. It was essential that at least two of the mutants used could be expected to show poor viability. On the other hand, the factors chosen could not be so deleterious that one could not expect to breed from them.

Extensive work involving the agouti locus had been done in Cambridge, of which Fisher's (1949*b*) experiment with the *A—un* interval was specifically concerned with the five agouti alleles yellow (A^Y), light-bellied agouti (A^L), dark-bellied agouti (A), tan belly (a^t) and non-agouti (a). These investigations showed that in general no significant inviability of any of the genotypes involving these alleles need be expected. Accordingly, no attempt was made to eliminate any of the four alleles (A^Y, A, a^t, a) available in the initial stocks used, although, by chance, most of the material involved a^t and a. In the single instance in which an Aa^t heterozygote ($a^t fiSd/A$) was available, in which either A or a^t may be regarded as dominant, A was treated as dominant and the heterozygote classed as "*Sd*-out". This was a matter of convenience, these heterozygotes being in short supply at that time. The decision to breed from this mating was fortunate since the progeny threw some light on the nature of the agouti locus (Wallace, 1954*b*).

Fidget was first described by Grüneberg (1943). To judge by his description, it is suitable in exhibiting impaired viability, but practically impossible to breed from homozygously. Grüneberg's more recent work (1952) re-states his earlier view on its infertility: "Some males are fairly reliable breeders; females often fail to breed; those that do have so far failed to rear their litters." Apparently no attempt was made to improve the performance of fidget in the intervening nine years, nor is it clear that this possibility has been realised by others working with him on this gene (Carter and Grüneberg, 1950, and Truslove, 1956). It is common experience that when a mutation first appears, it is usually deleterious in one or more respects: further, the variability of the effects of single genes is also well known, and the possibility of altering or increasing such effects by selection has been amply demonstrated. It seemed reasonable, therefore, to

suppose that a fixed degree of infertility and inviability of fidget was not necessarily characteristic of it. Accordingly it was introduced to several different stocks in Cambridge. The ratios then obtained confirmed the view that not only its viability and fertility, but also its expression are very variable and an attempt was made to improve its performance by selection.

Response was rapid, and a stock was maintained by homozygous and backcross matings by the time the three-point experiment was planned. Viability of fidgets in the three-point is such that, while in the whole experiment there is, as desired, a significant deficiency of fidgets, this deficiency is not too marked to make the gene impossible to work with, and there are several matings which produced large progenies with no shortage of fidgets : three, with progenies exceeding 30, produced a slight excess. (There is, however, no heterogeneity in the overall segregation of fidgets.)

Further, the fertility of both sexes became quite reliable, though still, as a whole, inferior to that of normals. The production of 549 progeny from male triple heterozygotes, all of which were mated to female fidgets, did not lag more than a few months behind the production of an equal number (550) from female heterozygotes and their male fidget mates. Of the 28 female fidgets used, 9 produced over 30 fully classified young, two of these over 50. (The calculated average output of 19·6 progeny per fidget mother is unduly low since many of them were not allowed to breed for more than a few weeks once the data for their particular type of mating were almost complete.)

A further doubtful point was clarified. Grüneberg (1943) had reported a relation between fidget and polydactylism which could have several explanations. This relation was shown not to be linkage, which might have complicated the three-point experiment, but rather the specific modification by fidget of the gene polydactyly (*py*). The work on fidget will be published in due course.

That Danforth's short tail (*Sd*) shows lowered viability was found by Dunn, Gluecksohn-Schoenheimer and Bryson (1940), who also established its genetic basis as a single major gene producing three distinct phenotypes. The heterozygotes have short tails of variable length, and the homozygotes are almost or completely tailless, and die shortly before or after birth. Variability of tail length and viability of both heterozygotes and homozygotes was established by Dunn *et alii* and by Fisher and Holt (1944) ; the latter also demonstrated rapid response to selection for large and small tail length in heterozygotes, accompanied by correlated changes in viability.

Experience in the manipulation of this factor was gained while conducting a continuation of Fisher's experiment. One of the aims of this experiment was to make *Sd* as completely recessive as possible, and this end had been largely achieved in the production in some matings of tails as long in the heterozygote as in the normal, and in the production of two proved homozygotes which lived, one for

7 days and the other for 22 days. The present writer produced in addition an animal, which, in the stock selected for long tails, was judged by reason of its very short tail, to be probably a homozygote : it lived for 40 days.

Experience showed that, while the expression of *Sd* is variable, it should not be difficult to classify on a comparatively unselected background ; and that, while its viability is also variable, it is controllable. One could be confident that, given a fairly favourable background, the mere inevitable selection of future matings from viable and fertile heterozygotes would ensure a sufficiently high and stable degree of viability.

Danforth's short tail and the four agouti alleles were derived from several sources, and the fidget mice from the investigation of its association with polydactyly. From these stocks, appropriate preparation matings were made to produce the four types of heterozygote of each sex :

(i) Tricoupling . . $\frac{a^t + Sd}{a\,fi +}$

(ii) a^t-out . . . $\frac{a + Sd}{a^t\,fi +}$

(iii) *Sd*-out . . . $\frac{a^t + +}{a\,fi\,Sd}$

(iv) *fi*-out . . . . $\frac{a^t\,fi\,Sd}{a + +}$

The first, tricoupling, is so named because each of the three factors is in coupling with each of the others. The other three heterozygotes have only one pair in coupling and two in repulsion ; each has a different pair in coupling, so exhausting all possible arrangements of two alleles at each of three loci. A convenient nomenclature is to use for the identification of each of these three heterozygotes a symbol for that pair of alleles which is displaced, *i.e.* " out ", as compared with tricoupling.

The four types of heterozygote were mated to triple recessives *a fi* (or $a^t\,fi$ where stated below) and 1099 progeny were bred ; half of these were from male heterozygotes and half from female, and they comprised about 135 from each type of heterozygote of each sex.

4. ANALYSIS

The full data are tabulated by genotypes in table 1 (Appendix).

(i) *Viability ratios*

It has been pointed out that both *fi* and *Sd* were expected, when the experiment was started, to show unequal survival of the various genotypes which are formed from them ; and that the members of

the agouti series were expected to have about equal viability. A summary of the three single-factor ratios (table 2, appendix) shows that these expectations are fulfilled, the ratios being as follows :

	$a^t : a$	$fi : +$	$Sd : +$
from female heterozygotes . .	272 : 278	235 : 315	241 : 309
from male heterozygotes . .	269 : 280	226 : 323	246 : 303

Sd and *fi* show significant losses : *Sd* has a survival rate approximating to 80 per cent., and *fi* to 72 per cent. The segregation of the agouti alleles is very insignificantly unequal.

There is clearly no significant difference between these ratios according to whether the offspring are derived from female or from male heterozygotes.

There is, however, significant heterogeneity in the ratios $+ : fi$ and $Sd : +$ within the progeny from the four types of female heterozygote, but no heterogeneity within the progeny from male heterozygotes. (χ^2 values for females are 9·9271 and 14·8115, each for 3 d.f., and for males 5·2813 and 5·8819, also for 3 d.f.) This suggests the possibility of a maternal effect. However, further analysis (Wallace, 1954*a*) does not give more than subsignificant support for this hypothesis. It merely suggests two possibilities : a difference in the performance of different heterozygous types corresponding to differences in the gene arrangement at the loci studied here, which constitutes a kind of position effect, and the confinement of these differences to the performance of female as against male heterozygotes, which in turn constitutes a kind of maternal effect.

Such possibilities, which may be explored by the analysis of the single-factor ratios and of ratios within complementary pairs, should not be overlooked. The balanced backcross provides very suitable material for this kind of investigation.

(ii) *Viability disturbance*

Viability disturbance may be defined as imperfect viability of the complementary pairs of progeny produced by the various modes of gamete formation, which is differential as between one mode and another.

It is clear that if, in a linkage experiment, the members of a series of alleles at only one locus have unequal viability, the whole data will not in this sense be disturbed ; but if the alleles at two or more loci have unequal viability, the survival rates of the different complementary pairs will be unequal.

The single-factor ratios (table 2, appendix) and the ratios within complementary pairs (table 3, appendix) show that *fi* and *Sd* are likely to disturb linkage data in this way, although, as will become manifest, further analysis is required to prove whether they actually do so or not.

Two of the single-factor ratios, as already mentioned, demonstrate unequal viability; and, further, they exhibit heterogeneity at least in the progenies from female heterozygotes. The members of the complementary pairs also are unequally viable, the total χ^2 (table 3B) being 43·7271 for 4 d.f., probability $<0{\cdot}001$; and there is, again, significant heterogeneity within complementary pairs from female heterozygotes, χ^2 for the segregation of *fiSd* : + (table 3B) being 8·9812 for 3 d.f., probability 0·05 to 0·02. (The a^t locus is ignored in table 3B since it is shown in table 2 to have no inequalities : table 3A gives an analysis for female heterozygotes which takes account of the a^t segregation.)

TABLE 4

Observed frequencies of complementary pairs (a)

Types of heterozygote	A : Progeny from female heterozygotes					B : Progeny from male heterozygotes				
	Modes of gamete formation					Modes of gamete formation				
	0	1	2	12	Totals	0	1	2	12	Totals
Tricoupling . .	65	43	21	7	136	71	36	25	3	135
a^t-out . . .	69	46	21	7	143	77	25	28	7	137
Sd-out . . .	71	41	15	7	134	71	39	24	5	139
fi-out . . .	66	36	25	10	137	71	27	33	7	138
Totals . .	271	166	82	31	550	290	127	110	22	549

Modes of gamete formation :
0 = no recombination.
1 = recombination in segment 1 (a^t-*fi*) and not in 2
2 = recombination in segment 2 (*fi*-*Sd*) and not in 1.
12 = simultaneous recombination in segments 1 and 2.

Complementary pairs form a 4×4 Latin Square :

A = a^t *Sd*, *a fi*	A B C D
B = *a Sd*, a^t *fi*	B A D C
C = a^t, *a fi Sd*	C D A B
D = *a*, a^t *fi Sd*	D C B A

Now, recombination estimates based on balanced data are rendered less precise than they would otherwise be if there is an appreciable change in the survival rate of one or more complementary pairs as between one type of mating and another. If single-factor ratios change during the course of the experiment, or if the ratios between members of complementary pairs change, one may in general expect the survival rates of the complementary pairs themselves to change. But the latter changes need not be as large as the former owing to the averaging effect of regarding the complementary pair as one unit. Nor do they necessarily follow at all, since the depletion of one genotype may conceivably be accompanied sometimes by an improvement in the viability of its complementary. Thus, although examination of single-factor ratios and of ratios within complementary pairs has shown such changes (at least in progeny from female heterozygotes), they need not on this account be regarded as proof of disturbance.

If there is differential viability of the complementary pairs, through this or any other cause, there will be non-proportionality within the observed numbers of the complementary pairs as produced by the various modes and the various types of heterozygote. A

TABLE 5

χ^2 test for 9 d.f. for viability disturbance between complementary pairs

	Expected frequencies (m)									
	A : Progeny from female heterozygotes					B : Progeny from male heterozygotes				
	Modes of gamete formation					Modes of gamete formation				
	0	1	2	12	Totals	0	1	2	12	Totals
Tric. .	67·0109	41·0473	20·2764	7·6655	136	71·3115	31·2295	27·0492	5·4098	135
a^4-out .	70·4600	43·1600	21·3200	8·0600	143	72·3679	31·6922	27·4499	5·4900	137
Sd-out .	66·0255	40·4436	19·9782	7·5527	134	73·4244	32·1548	27·8506	5·5701	139
fi-out .	67·5036	41·3491	20·4255	7·7218	137	72·8962	31·9235	27·6503	5·5301	138
Totals .	271	166	82	31	550	290	127	110	22	549
	$(a-m)^2/m$									
					Total χ^2					Total χ^2
Tric. .	0·0603	0·0929	0·0258	0·0578	0·2368	0·0014	0·7287	0·1552	1·0734	1·9587
a^4-out .	0·0303	0·1869	0·0048	0·1394	0·3614	0·2965	1·4131	0·0110	0·4153	2·1359
Sd-out .	0·3748	0·0077	1·2405	0·0404	1·6634	0·0801	1·4572	0·5324	0·0583	2·1280
fi-out .	0·0335	0·6920	1·0245	0·6721	2·4221	0·0493	0·7593	1·0350	0·3907	2·2343
χ^2 sub-totals	0·4989	0·9795	2·2956	0·9097	4·6837	0·4273	4·3583	1·7336	1·9377	8·4569
Degrees of freedom					(9)					(9)

preliminary test for disturbance is, then, the χ^2 test for 9 degrees of freedom ; this should detect gross non-proportionality ; it is made by comparing the observed frequencies of the 16 complementary pairs with those expected on the basis of the marginal totals (tables 4 and 5).

The two χ^2 values, both less than 9, are insignificant for their 9 degrees of freedom.

A more discriminating test for disturbance is obtained by taking out the 3 degrees of freedom relating to the totals of each complementary pair (table 6 ; the observed frequencies are obtained by addition from table 4, and the expected from table 5). These three degrees of freedom relate to overall viability disturbance, leaving six for detecting changes in the survival rates of complementary pairs, *i.e.* variation in differential viability.

These χ^2 values are extremely low and contribute very little to the χ^2 for 9 d.f.

Neither of these tests, however, is specific for viability disturbance, and it may be that some of the variations in numbers of the different pairs of genotypes have been compensated in adjusting the expectations from the row and column totals. An exact and specific treatment is given by Fisher (1949*a*). When applied to the present data, χ^2 for 3 d.f. for female heterozygotes is 0·23475 and for male heterozygotes is 3·280925. These values are only very slightly greater than those obtained in table 6.

The insignificance of these χ^2 values points to the conclusion that there is no significant viability disturbance in either body of data.

TABLE 6

χ^2 test for 3 d.f. for viability disturbance between complementary pairs

Genotypes	A. Progeny from female heterozygotes			B. Progeny from male heterozygotes		
	Observed (a)	Expected (m)	$(a-m)^2/m$	Observed (a)	Expected (m)	$(a-m)^2/m$
$a^t Sd$; fi . .	136	137·8709	0·0254	127	136·3844	0·6457
Sd ; $a^t fi$ . .	144	139·4855	0·1461	151	136·8178	1·4701
a^t ; $fiSd$ . .	135	135·7110	0·0037	130	137·8871	0·4511
$a^t fiSd$; a .	135	136·9327	0·0273	141	137·9107	0·0692
Totals .	550	549·9991	0·2025 ($n = 3$)	549	549·0000	2·6361 ($n = 3$)

The remaining values of χ^2 for 6 d.f. (4·4489 for progeny from female heterozygotes and 5·1760 for male) are also insignificant, and indicate that, although there are significant changes in the output of the four kinds of female heterozygote in the single-factor ratios, and in the ratios between members of pairs involving the *fi* and *Sd* loci, these changes have produced no appreciable change in the overall survival rates of the complementary pairs.

The lack of disturbance need not be regarded as rendering the balanced design unnecessary. That the inequality between the survival rates of the two complementary pairs involving *fi* and *Sd* would be insignificant was not known when the experiment was planned. Moreover, this inequality is sufficient to give estimates of the recombination value *fi-Sd* which differ as between coupling data and repulsion, though insignificantly so : clearly the estimate based on the combined data is more accurate than one based on either separately. Further, it could not be certain, when planning the experiment, that the almost complete equality of viability within the agouti alleles which had so far been experienced would be repeated on the genetic background on which this experiment was

carried out ; nor, further, could it be certain that any slight inequality which did exist would not, when combined with the inequalities already observed within the *fi* and *Sd* pairs of alleles, produce significant heterogeneity in the survival rates of the pairs of complementaries made up by all three loci. Hence the balanced design was a necessary safeguard.

In conclusion, analysis of the data from female and from male heterozygotes, treated separately, shows that there is evidence for slight differential viability : hence the high degree of balance attained is justified. The disturbance, however, is insignificant in both sexes : hence the addition formula for the estimation of recombination values is sufficiently accurate. Because of the very mild degree of disturbance, the data are not ideal for a demonstration of Fisher's method of correcting for strong differential viability. The disturbance from

TABLE 7

2xn table of frequencies of modes of gamete formation

	0	1	2	12	Totals
Heterozygous ♀♀ . .	271	166	82	31	550
,, ♂♂ . .	290	127	110	22	549
Totals . . .	561	293	192	53	1099

(Corrected) χ^2 for 3 d.f. = 11·4461
Probability = 0·01 to 0·001

female heterozygotes is less than that from males, despite a sub-significant maternal effect of the heterozygotes on the viability of genotypes involving *fi* and *Sd*.

(iii) *The effect of sex on recombination*

That there are differences in the frequencies of the modes of gamete formation according to the sex of the heterozygous parent can most readily be seen from the χ^2 value obtained from the *2xn* table above : (table 7 : derived from table 4)

These differences, however, are not distributed uniformly along the whole segment *a*^{t}*-Sd*, for the adjacent segments *a*^{t}*-fi* and *fi-Sd* behave dissimilarly (table 8).

Since twice the standard error of the difference between the recombination fraction from female and from male heterozygotes does not exceed the difference in the segment *a*^{t}*-fi*, this difference is significant at the 5 per cent. level of probability. The difference in the *fi-Sd* segment is in the opposite direction, that is the recombination value is larger in the males here whereas it is larger in the females for *a*^{t}*-fi* : but this difference, by the same criterion, is not significant.

Nevertheless, the fact that it is in the opposite direction indicates that there may be an abrupt change at about the locus of *fi* between one segment and the other in the response to a difference of sex. Such an abrupt change has been noted twice in the mouse ; first at

TABLE 8

Effect of sex on recombination value

Segment	Sex of het. parent	Recomb. fraction	Standard error (per cent.)	Difference between r.fs. (per cent.)	Twice s.e. of difference (per cent.)
a^t-*fi*	♀	35·8182	± 2·0445	8·68	5·58
	♂	27·1403	± 1·8979		
fi-Sd	♀	20·5454	± 1·7228	3·50	5·02
	♂	24·0437	± 1·8240		
a^t-*Sd*	♀	45·0909	± 2·1217	...	...
	♂	43·1694	± 2·1140		

the locus of *undulated* in this same chromosome (Fisher and Landauer, 1953) and recently at the sh_2 locus of linkage group VII (Michie, 1955). Adding the data for the interval *a-un* given by Fisher (1949*b*), and that from the balanced four-point backcross on which interim reports have so far been given (Fisher and Landauer, 1953, and Owen, 1953*a*), we have the following table (table 9) :

TABLE 9

Effect of sex on intervals a-un-we-pa

a-un		*un-we*	*we-pa*	
♀♀ 4·7237±0·33 per cent.	from 8576 progeny	7·39±0·52	4·20±0·40	from 5002 progeny
♂♂ 4·6753±0·32 per cent.		4·44±0·41	2·24±0·30	

It is not possible to discern from the present three-point whether all of the sex effect observed in the segment *a*^{t}-*fi* is due to the effect noted earlier in the *un-pa* segment, but future data from the current *Ra-a-we-fi-Sd* experiment may allow of further analysis.

It is hoped that this five-point may also show significantly the tendency observed in the three-point for a higher recombination value for *fi-Sd* in the male. Of the three published cases in which recombination in the male is significantly higher than in the female, the most extreme is that reported for the segment *bt-Ca-N* (Mallyon, 1951) : the recombination fraction is nearly twice as great for *Ca-N* in males as in females and about three times as great for *bt-Ca*. A convenient table comparing recombination in the two sexes was compiled in 1953 (Michie, 1955) ; the additional notes given here bring it up to date for linkage groups V and VI.

(iv) *The effect of age on recombination*

Very little attention has so far been paid in the literature to age effects in the mouse. Nor does it appear to be generally realised that real effects of age and sex on recombination must be taken into account in the treatment of data for mapping purposes and in the study of interference.

The only balanced experiment large enough to show these effects is that reported by Fisher (1949*b*). Here, the interval *a-un* shows a decrease in recombination value with increasing age; a stronger decrease is found in male gametogenesis than in female, but this difference is not found to be significant. The even larger experiment referred to by Fisher and Landauer (1953) and Owen (1953*a*), however, does not confirm this age effect (Owen and Darroch, unpublished).

There is no easy explanation for this inconsistency, and further extensive data are needed. In running the present three-point experiment, it was hoped that some contribution on this subject might be forthcoming, and, towards the end of the work, matings which had already run for several months were allowed to continue as long as possible after the records were closed on completion of "balance". The total number of progeny included in the data therefore exceeds the 1099 bred for "balance", being 766 from female heterozygotes and 591 from male (table 10).

The test of significance used in table 10 is that given by Fisher (1925-54). In addition to the symbolism given in the table, we have :

$$q = S(a')/S(n), \quad p = S(a)/S(n)$$
$$S(t \times a') = A' ; \quad S(t \times a) = A ;$$
$$S(t-\bar{t})^2 = S(t^2 n) - \frac{\{S(tn)\}^2}{Sn}$$

then

$$\chi^2 = \frac{(qA - pA')^2}{pq\, S(t-\bar{t})^2}$$

$(qA-pA')$ is positive if the recombination value increases with maternal age, and negative if it decreases.

It is interesting to note that the only segment which shows a significant response to age ($\chi^2 = 5{\cdot}2162$ for 1 d.f.) is the one which also shows a significant response to sex, namely *a^t-fi*. This segment shows higher recombination in the female than in the male, but, while this value remains almost steady in females with advancing age, it increases in males with advancing age. Since one end of this segment, namely *a-un*, showed in one body of data (Fisher, 1949*b*), if not in the later body, a decrease for both sexes with advancing age, the reality of any age effect is very doubtful.

If the evidence for the segment *a^t-fi* is taken at face value it appears possible that there is a real response to age, but because the

TABLE 10

Test for regression of recombination value on age of heterozygous parent

A : Progeny from female heterozygotes

Age of parent (months) (t)	Total no. of offspring (n)	a^t-fi			fi-Sd			a^t-Sd		
		N (a')	R (a)	R.f. %	N (a')	R (a)	R.f. %	N (a')	R (a)	R.f. %
0-2	28	14	14	50·00	25	3	10·71	11	17	60·71
2-3	87	52	35	40·23	65	22	25·29	42	45	51·72
3-4	88	61	27	30·68	67	21	23·86	46	42	47·73
4-5	98	66	32	32·65	79	19	19·39	61	37	37·76
5-6	103	69	34	33·01	78	25	24·27	58	45	43·69
6-7	69	42	27	39·13	56	13	18·84	39	30	43·48
7-8	55	39	16	29·09	45	10	18·18	33	22	40·00
8-9	75	45	30	40·00	61	14	18·67	39	36	48·00
9-10	56	44	12	21·43	43	13	23·21	35	21	37·50
10-11	39	26	13	33·33	33	6	15·38	20	19	48·72
11-12	28	15	13	46·43	22	6	21·43	17	11	39·29
12-13	16	11	5	31·25	15	1	6·25	10	6	37·50
13+	24	14	10	41·67	22	2	8·33	14	10	41·67
	766 $S(n)$	498 $S(a')$	268 $S(a)$		611 $S(a')$	155 $S(a)$		425 $S(a')$	341 $S(a)$	
Deviation		−18·70786			−54·12330			−63·47126		
χ^2 for 1 d.f.		0·2109			2·4876			2·2356		

All recombination fractions decrease with increase in age. None shows a significant trend.

Note.—N = non-recombinants, R = recombinants.

B. Progeny from male heterozygotes

Age of parent (months) (t)	Total no. of offspring (n)	a^t-fi			fi-Sd			a^t-Sd		
		N (a')	R (a)	R.f. %	N (a')	R (a)	R.f. %	N (a')	R (a)	R.f. %
0-2	16	11	5	31·25	11	5	31·25	8	8	50·00
2-3	72	50	22	30·56	54	18	25·00	34	38	52·78
3-4	87	68	19	21·84	73	14	16·09	60	27	31·03
4-5	112	94	18	16·07	77	35	31·25	65	47	41·96
5-6	78	59	19	24·36	67	11	14·10	48	30	38·46
6-7	67	48	19	28·36	53	14	20·90	40	27	40·30
7-8	37	22	15	40·54	26	11	29·73	19	18	48·65
8-9	38	25	13	34·21	29	9	23·68	18	20	52·63
9-10	36	21	15	41·67	23	13	36·11	18	18	50·00
10-11	29	20	9	31·03	20	9	31·03	13	16	55·17
11-12	12	9	3	25·00	9	3	25·00	8	4	33·33
12-13	4	3	1	25·00	4	0	...	3	1	25·00
13+	3	1	2	66·67	2	1	33·33	2	1	33·33
	591 $S(n)$	431 $S(a')$	160 $S(a)$		448 $S(a')$	143 $S(a)$		336 $S(a')$	255 $S(a)$	
Deviation		+64·37839			+24·32828			+22·99621		
χ^2 for 1 d.f.		5·2162			0·8018			0·5357		

All recombination fractions increase with increase in age. Only a^t-fi shows a significant trend.

Note.—N = non-recombinants, R = recombinants.

data is not distributed according to age in the same fashion in progeny from females as from males, the whole, or part, of the observed difference in the overall response to sex is only apparent. One interpretation of this latter situation is represented in fig. 1, where straight regression lines have been fitted by eye to the observations given in fig. 2. Support for this latter hypothesis is found in the fact that the mean age of the males is considerably lower than that for females, these being 5·1726 and 5·8399 months respectively (mean age = $\frac{S(t \times n)}{S(n)}$). The expected recombination fractions corresponding to these ages (fig. 1) are 27 and 35 per cent. respectively; that is, the hypothetical lines give at these ages a close approximation to the observed average values.

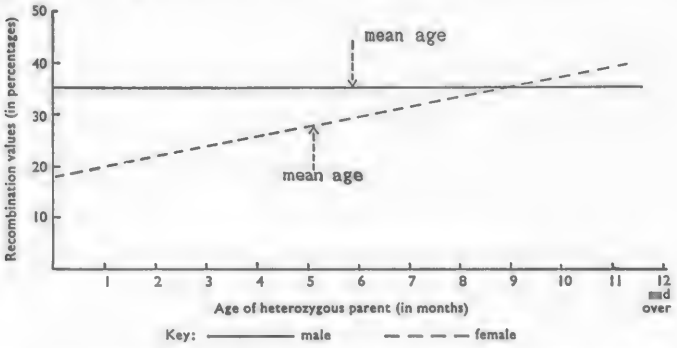

FIG. 1.—Hypothetical trends of regression of ♂ and ♀ recombination values on age (a^t-fi).

However, an exact fit has not been calculated because it appears unlikely that the relation is, in fact, one of straight line regression. A curved line is often found to give a better fit to biological data of this sort. Moreover, if the relation is assumed to be what the observations more readily suggest, namely a U-shaped curve, some explanation may be found for the conflicting results so far obtained for the *a-un* segment.

Fig. 2 shows the observed values for the *a-un* and a^t-*fi* intervals at corresponding ages as found by Fisher, by Owen and Darroch, and by myself respectively. The data for nine months and over are included, but they are rather scanty in all the bodies of data and may therefore be unreliable. It is striking that while Fisher's observations for *a-un* give a good agreement with the assumption of a steady decrease, the recombination fractions at six months are higher than those for four months—as they are also in Owen's data for the same segment and my own for the a^t-*fi* segment. In fact, it is clear that, disregarding the data for nine months and over, a U-shaped curve would give a good fit.

It is possible, therefore, that the whole segment *a-fi* shows a fall in recombination during the first four or five months, and a rise (though probably a shallow one) for the next few months. Conceivably the

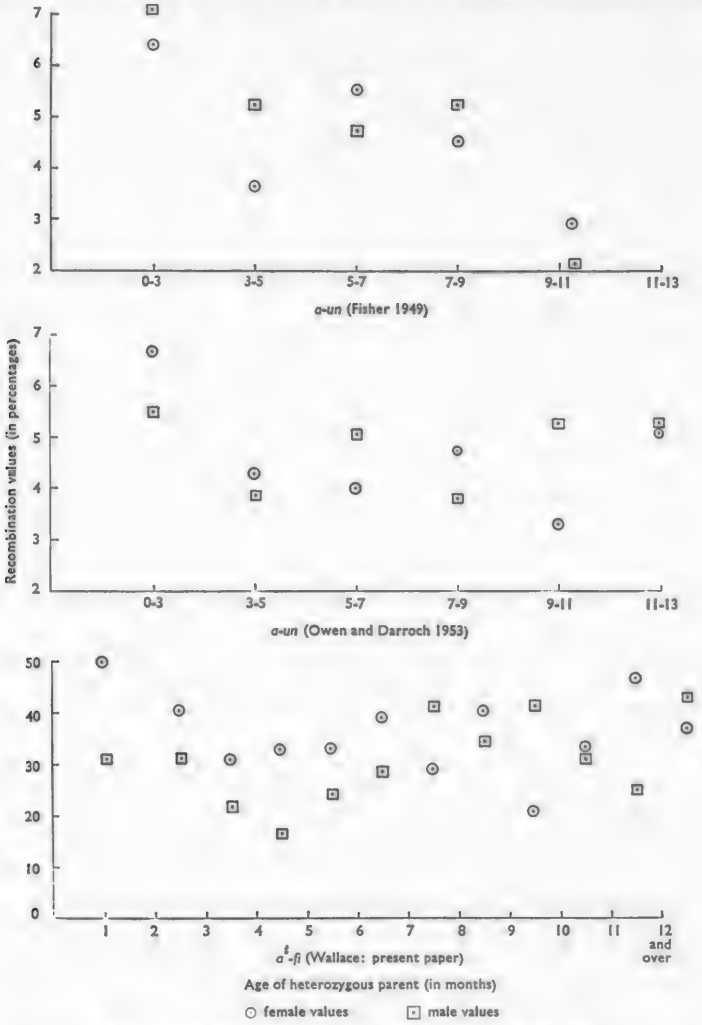

FIG. 2.—Observed recombination values plotted against age.

fi-Sd segment also shows a very slight response which might be discernible in more extensive data. In fine, the degree of response of the whole chromosome may be different for different segments ; and there may —or may not—be a continuous rather than an abrupt change in

degree throughout its length. At present one cannot say more than that the agouti end appears to respond more strongly than does the *Sd* end.

This age response may be more or less different as between male and female gametogenesis. If so, it is possible that the difference in mean age for the two sexes at which the three-point data are drawn gives an unreal magnitude to the difference in sex for *a-fi* as calculated without regard to age. The magnitude of the sex difference in the *un-we-pa* segments may also be inflated in this way ; but since there is no appreciable sex difference for the *a-un* interval in either Fisher's or Owen's data, it seems likely that there is a real change in response to sex at about the *un* locus.

Finally, it is clear from fig. 2 that the assumption of a U-curve may resolve the apparent conflict in the data for the *a-un* segment : the later data show a rise in the second " arm " of the curve (up to 9 months) more strongly than does the earlier, and thus does not reflect well the latter's overall decrease of recombination with age. Methods of fitting various curvilinear regressions to the observed recombination value, or to its corresponding value as obtained by angular transformation (such as that used by Fisher in 1949), will be elaborated when more extensive data are available.

At present, the significance of the age effect for *a-fi* is not large enough to warrant the labour of correcting for it in the estimation of recombination values and in the treatment of interference in male and female gametogenesis. It will, however, be assumed that there is a real sex difference, and data from male and female heterozygotes must accordingly be treated separately for mapping purposes. Provision is being made in the current five-point experiment for the distribution of data according to age to be as even as possible, and a more thorough examination of the effects of both age and sex on recombination will be made.

(v) *The effect of age on the sex-ratio*

An interesting anomaly emerges from the data compiled for the effect of age on recombination. This is a change according to maternal age in the sex-ratio of offspring produced both by fidget mothers and by the aggregate of the four kinds of heterozygous mothers.

The data are divided into two parts : offspring from females of from two to ten months, and offspring from females of over ten months. This arrangement is more a matter of convenience than of choice, for the data were not compiled specifically for an investigation of this sort. The numbers from mothers over ten months are rather low, and it may be that if there were more data from the later ages and if they were divided according to the ages of the mother in months, a progressive change would be discernible. As they stand, the two bodies of data (A and B, table 11) give a subsignificant χ^2 for disproportionality between the two sex-ratios, the direction of this

disproportionality being the same in both cases; and these, when combined (C, table 11) give a significant χ^2 value.

Changes in the sex-ratio of inbred lines and of F_1 from reciprocal crosses between them have been observed by Howard, McLaren, Michie, and Sander (1955); they found a significant rise in the sex-ratio with increase in litter size; this is interpreted as due to a greater vulnerability of the female fœtus over the male, resulting in a greater depletion of females where competition *in utero* is greatest. They found that parity has no effect.

TABLE 11

Change in sex-ratio from mothers under 10 months old compared with those over 10 months

(Only data from ♀♀ mated at 2 months and living until 10 months old or later are included)

	A. Progeny from fidget females			B. Progeny from heterozygous females			C. Progeny from all females		
	♀	♂	Total	♀	♂	Total	♀	♂	Total
<10 m.	199	164	363	205	179	384	404	343	747
>10 m.	24	29	53	37	50	87	61	79	140
	223	193	416	242	229	471	465	422	887
Contingency χ^2_c(1 d.f.)		1·3299			2·9262			4·8103	

It seems unlikely that the rise in the sex-ratio of progeny from females over 10 months observed in the present data, is correlated with an increase in litter size; for general experience, and published work (*cf.* Murray, 1934), indicate that litter size decreases from a maternal age of about eight months onwards: the mean litter size for females over 10 months old is therefore probably lower than that for younger females. The assumption of Howard *et alii* that the female sex is more subject to higher pre-natal mortality is, as they point out, in conflict with the views of several other workers: that the male sex is the more vulnerable is more harmonious with the present observations and with the assumption that intra-uterine competition is an important influence on the natal sex-ratio. On the other hand, the present observations may be more explicable in terms of parity than of litter size, a possibility which will be tested if the larger data from the five-point confirm the present findings.

5. INTERFERENCE AND MAPPING

(i) *Interference: some historical notes*

The term "interference" was first coined by Muller (1916). It may be defined as the influence of one crossover upon others in the

same strand. Both cytological and genetic evidence shows that it is usually positive, *i.e.* the occurrence of one crossover tends to inhibit the occurrence of others near it.

It was evident as early as the second decade that interference varies from organism to organism and even from one segment of a chromosome to another. Several crude measures of the strength of interference were adopted, including that known as " coincidence ". Although there was a verbal similarity between the various definitions of this term, it covered, during the next two decades, at least three different quantities. Stevens (1936) was responsible for defining it properly for the first time and for giving it concise mathematical formulation ; this can be expressed as follows : the index $C = z/y_1 y_2$ where y_1 and y_2 are the observed recombination fractions in segments 1 and 2, and z is the observed frequency of double recombinants, *i.e.* recombinants arising from two simultaneous crossovers in segments 1 and 2. Haldane's early attempts (1919) to include both multiple crossing-over and interference in the general theory of crossing-over, give C an approximate value of $\frac{1}{2}$ for segments of intermediate length, and assume a uniform degree of interference along the chromosome. But " coincidence " is not a very satisfactory measure since it depends on the size of y_1 and y_2 and therefore cannot be constant.

Other theoretical work and practical application was done by several workers, notably by Jennings (1923) and by Anderson and Rhoades (1931). But none of the mapping functions constructed by these authors was found to have more than a limited application, and it was not until 1944 that a relationship of more universal application and practical value was found. Kosambi put forward the functions

$$x = \tfrac{1}{2} \tanh^{-1} 2y \quad \text{and} \quad y = \tfrac{1}{2} \tanh 2x,$$

and the corresponding simple addition theorem for y :

$$y_{1+2} = \frac{y_1 + y_2}{1 + 4y_1 y_2}.$$

Owen (1950), following a suggestion made by Sir Ronald Fisher, has proposed the term " Kosambi coefficient ", for a value K comparable to the coincidence ratio C : unlike C, however, it can, on theoretical grounds, be expected to be constant for segments of any length. Its relation to C may be written : $K = C/2y_{1+2}$, where y_{1+2} is the observed recombination value over segments 1 and 2. The Kosambi relations are satisfied if, and only if, a certain intensity of interference operates uniformly over the total length of the linked segments.

Kosambi's mapping function has been found to give a good fit to many cases of three- and four-point data, including Haldane's 1919 data and Jodon's data (Bhat, 1950). The Kosambi relation is not, however, completely satisfactory. Firstly, as has been pointed out, it assumes a certain uniform intensity of interference over the

length of the chromosome : it takes no account of the position of loci in relation to the centromere and terminus, both of which have been shown to affect the intensity. Secondly, it does not provide a complete theory of recombination, for it does not specify the frequencies of the modes of gamete formation for more than three loci simultaneously. And finally it sets an upper limit of 50 per cent. to recombination between any two linked loci. The importance of the first two defects is obvious : the third could not have become important unless cases of more than 50 per cent. recombination were shown convincingly to occur.

Further theoretical advance was not made until the half-century. It is true that Mather (1933, 1936 and 1937) did much to fill the gaps in the cytological and genetic spheres, and he prepared a theory of serial formation of chiasmata based on Jennings' ideas (1923), which will be remembered as an important phase in the development of recombination theory. But no doubt a more elaborate formal analysis was not an attractive proposition so long as there was no genetical evidence which strikingly conflicted with current thought. Experimental data involving three or more loci, to which Kosambi's formulæ can be applied, have not been abundant nor very free from disturbance, and hence a sufficient number of cases which it has not fitted have been lacking. Moreover, although there have been reports of recombination exceeding 50 per cent.—Clausen (1926) in *Viola*, Wellensiek (1929*a* and *b*) in *Pisum*, and Fisher and Mather (1936*a*, *b*) in mice—contemporary thought has judged them to have been either somewhat biassed or not sufficiently significant to be completely convincing. They also lack cytological confirmation. For values exceeding 50 per cent. require the occurrence of chromatid interference ; this involves a non-random relationship between the two strands exchanging material at one point and the two which exchange it at a second point. It alone provides the condition necessary for recombination exceeding 50 per cent., namely that the chance of a single strand being involved in an odd number of chiasmata is in excess of its chance of being involved in an even number. Cytological evidence on this point is very sparse and difficult to obtain ; as far as it goes, it supports only a mild degree of chromatid interference (Newcombe 1941 and Huskins and Newcombe 1941).

It was the experiment with $wavy_2$, $shaker_2$ and sex (Wright, 1947) which produced not only strong evidence for more than 50 per cent. recombination, but unusual mutual relationships between the three markers involved ; and this called for a more complex theory of interference than any currently accepted.

These data and the earlier data reported by Fisher and Mather (1936*a*, *b*) were unique in being, through careful design, almost completely free from disturbance through differential viability, and were therefore more convincing than earlier claims. Together they provided a stimulus, and the three-point data provided also a framework, for the further development of recombination theory. Fisher,

influenced to some extent by Mather's concept of chiasma formation as a serial process, produced a new general theory of wide applicability (Fisher *et al.*, 1947, and Fisher, 1948 and 1951). These ideas were developed in greater detail by Owen (1949*a*, *b*, 1950, 1951, 1952, 1953*a*, *b*) ; he showed how finite lengths of chromosome arms may be taken into account, and indicated that recombination fractions exceeding 50 per cent. are characteristic of a wide range of intercept distribution functions provided interference is sufficiently strong.

These latest developments of the theory of recombination and the mathematical models which form an integral part of them now

TABLE 12

Test of significance of interference

	A. In female gametogenesis *fi-Sd*			B. In male gametogenesis *fi-Sd*			C. ♂ and ♀ combined *fi-Sd*		
	Cross-overs	Non-cross-overs	Total	Cross-overs	Non-cross-overs	Total	Cross-overs	Non-cross-overs	Total
a^{+}-*fi*									
Crossovers . .	31	166	197	22	127	149	53	293	346
Non-crossovers .	82	271	353	110	290	400	192	561	753
	113	437	550	132	417	549	245	854	1099
Contingency χ^2_c for 1 d.f.		3·9022			8·9558			13·6008	

require suitable genetic material against which they can be tried, tested and perhaps modified. Owen (1948) has tested some of his models, using *Drosophila* data, and found good agreement for chromosomes II and III : the data as a whole give a completely convincing fit despite the failings already mentioned. *Drosophila* has certain genetic features dividing it rather sharply from the rest of the animal kingdom and from plants (such as the suppression of crossing-over in the male), so that there is no *a priori* reason for expecting the same kinds and degrees of inter- ference in other organisms as are found in *Drosophila*. Further experiments of the kind here described, using a large range of organisms, are needed in order to provide the testing material for interference theory.

(ii) *The detection of interference*

A simple test of the significance of interference between any two segments in multi-point data is a χ^2 test based on 2×2 tables of the observed gametic output. In the present data, interference in both male and female gametogenesis is beyond doubt (table 12).

(iii) *The measure of Interference*

It has been stated that " coincidence " is a useful measure for obtaining an idea of the order of magnitude of interference ; it retains a place among related statistical tools, since it does not assume any theoretical intensity. Table 13 gives estimates of the value of C obtained by the application of the formula $\hat{C} = z/y_1y_2$ to the data as summarised in table 12.

TABLE 13

Values of " coincidence "

A. For female gametogenesis	$\hat{C} = 0{\cdot}7659 \pm 0{\cdot}11$
B. For male gametogenesis	$\hat{C} = 0{\cdot}6141 \pm 0{\cdot}11$
C. For males and females combined . .	$\hat{C} = 0{\cdot}6871 \pm 0{\cdot}08$

The value of $\hat{C}$ is in all cases less than unity ; hence interference is positive.

As Owen (1950) has shown, for short segments C is generally large near the centromere, and small near the terminus. However, it is also dependent on the length of the two segments compared, being lower for long segments : in the present case, therefore, it is unreliable as an indicator of the position of the centromere. Owen (1953*a*) has indicated that the latter is probably " moderately distant " from the smaller segments *a-un-we-pa*. It is reasonable, therefore, to test the hypothesis that the larger segment *a^t-fi-Sd* is roughly in mid-arm.

In this position, it may be expected to show interference at the Kosambi level. The measure K, which is analogous to C but is based on the particular degree of interference suggested by Kosambi, is equal to unity in mid-arm, and may be expected to rise near the centromere and fall near the terminus. A convenient way of testing the hypothesis of a Kosambi degree of interference is, therefore, to obtain an estimate of K and of its variance. The variance may be obtained by more than one method, a convenient one being available on testing the fit of a Kosambi map. In the present case, it is reasonable to expect a K value approximately equal to unity, and it is also instructive to obtain as reliable a map as current theory can supply. The procedure followed is, therefore, to fit a map for the observed frequencies of gamete formation assuming that $K = 1$, to test the goodness of fit, and to calculate the exact value and variance of K.

The method of fitting a Kosambi map is an example of Fisher's scoring technique (1946). The present particular application of it has been available for some six years as teaching material in the Cambridge Department of Genetics. Its publication in a practical form may be of general use.

(iv) *Fitting a Kosambi map*

(a) *Female gametogenesis.* Empirical estimates of the three recombination values are made as follows (from table 7) :

$$a^t\text{-}fi = \frac{(1)+(12)}{N} = 197/550 = 35{\cdot}8182 \text{ per cent.}$$

$$fi\text{-}Sd = \frac{(2)+(12)}{N} = 113/550 = 20{\cdot}5455 \text{ per cent.}$$

$$a^t\text{-}Sd = \frac{(1)+(2)}{N} = 248/550 = 45{\cdot}0909 \text{ per cent.}$$

To fit a Kosambi map, trial recombination estimates are made, based on Kosambi's addition formula ; and then adjustments are calculated, using the Maximum Likelihood Method, so that the three

TABLE 14

Provisional expectations for the four recombinant classes, and test of their fit with the observations

Mode	Obs.	Expectation	m_o	$\frac{(\text{Obs.-Exp.})^2}{\text{Exp.}}$
0	271	$1-y_1-y_2+y_{12} = 1-\frac{1}{2}(y_1+y_2+y_{1+2})$:	280·5859	0·3275
1	166	$y_1-y_{12} = \frac{1}{2}(y_1-y_2+y_{1+2})$:	159·4141	0·2721
2	82	$y_2-y_{12} = \frac{1}{2}(-y_1+y_2+y_{1+2})$:	76·9141	0·3363
12	31	$y_{12} = \frac{1}{2}(y_1+y_2-y_{1+2})$:	33·0859	0·1315
Totals	550	1 1	550	$\chi^2 = 1{\cdot}0674$

final estimates conjointly fit the empirical values with maximum precision. The latter estimates are then converted into map distances.

It is convenient, in order to shorten the calculation, to use trial estimates as near the final ones as can be judged by inspection. Empirical values for small segments are usually more accurate than those for large ones, so a convenient approximation to these may be taken as trial values. A good trial value for the largest segment is supplied by Kosambi's addition formula. We take

$$a^t\text{-}fi = = 0{\cdot}35 = y_1$$

$$fi\text{-}Sd = = 0{\cdot}20 = y_2$$

$$a^t\text{-}Sd = (y_1+y_2)/(1+4y_1y_2) = 0{\cdot}4296875 = y_{1+2}$$

The adjustments, dy_1 and dy_2, are calculated from the variances and scores related to changes in y_1 and y_2, as follows :

Provisional expectations (m_o) for the four recombinant classes are made. These are based on the trial recombination estimates, and are calculated from the identity $y_{12} = \frac{1}{2}(y_1+y_2-y_{1+2})$ (table 14).

A preliminary χ^2 test is included. It tests the goodness of fit of the expectations based on Kosambi with the observed numbers. At 1·0674 for 1 degree of freedom, it is not significant. The expectations

based on adjusted values will in general improve the goodness of fit and have a smaller χ^2.

Next a computation table is made (table 15).

TABLE 15

Computation table

Class	Obs.	Exp. m_o	$\frac{dm_o}{dy_1}$	$\frac{d}{dy_1}(\log m_o)$	$\frac{dm_o}{dy_2}$	$\frac{d}{dy_2}(\log m_o)$
0	271	280·5859	−415·9914	−1·4825813	−360·6020	−1·2851751
1	166	159·4141	415·9914	2·6095017	−189·3980	−1·1880881
2	82	76·9141	−134·0086	−1·7423151	360·6020	4·6883731
12	31	33·0859	134·0086	4·0503247	189·3980	4·7244318

To fill up the computation table, calculate first $d(\log m_o)/dy_1$ for the double crossover frequency (12) as :

$$\frac{1}{y_1} + \frac{1}{y_1+y_2} - \frac{4y_2}{1\times 4y_1y_2}$$

Calculated directly, this is :

2·85714286	$1/y_1$
1·81818182	$1/(y_1+y_2)$
−0·625	$-4y_2/(1+4y_1y_2)$
4·05032468 (to 8 places).	

Similarly $d(\log m_o)/dy_2$ for (12), is calculated from the above equation, interchanging y_1 and y_2, and is as follows :

5·0
1·81818182
−1·09375
5·72443182

The first of these is multiplied by the value of m_o for (12) to give the value of dm_o/dy_1, *i.e.* $4{\cdot}05032468\times 33{\cdot}0859 = 134{\cdot}0086$. The remaining values for dm_o/dy_1 are obtained as follows :

The value for (2) is equal and opposite in sign to that for (12). The value for (1) = total, minus the value for (12),

$$= 550 - 134{\cdot}0086 = 415{\cdot}9914.$$

The value for (0) is equal and opposite to that for (1).

The remaining values for $d(\log m_o)/dy_1$ are obtained by dividing the values for dm_o/dy_1 for (0), (1), (2) by the corresponding expected m_o values.

The values of dm_o/dy_2 and $d(\log m_o)/dy_2$ are obtained by the same

process as those for dm_o/dy_1 and $d(\log m_o)/dy_1$; it must be remembered that for dm_o/dy_2 the value for (12) is equal and opposite to that for (1), and that for (2) is equal and opposite to that for (0).

From the computation table the terms of the Information Matrix

$$\begin{Bmatrix} \Sigma\left[\frac{dm_o}{dy_1}\left(\frac{d}{dy_1}\log m_o\right)\right] & \Sigma\left[\frac{dm_o}{dy_2}\left(\frac{d}{dy_1}\log m_o\right)\right] \\ \Sigma\left[\frac{dm_o}{dy_2}\left(\frac{d}{dy_1}\log m_o\right)\right] & \Sigma\left[\frac{dm_o}{dy_2}\left(\frac{d}{dy_2}\log m_o\right)\right] \end{Bmatrix}$$

which may be abbreviated as

$$\begin{pmatrix} I_{11} & I_{12} \\ I_{12} & I_{22} \end{pmatrix}$$

are obtained by multiplying the entries in the fourth and fifth columns, and adding the products for all rows, and repeating the process for the fifth and sixth columns and the sixth and seventh.

The computation table also provides the scores, S_1 and S_2,

$$\Sigma\left[\text{obs}\times\frac{d}{dy_1}\log m_o\right] = S_1 \qquad \Sigma\left[\text{obs}\times\frac{d}{dy_2}\log m_o\right] = S_2$$

for the two adjustable variables y_1 and y_2. These scores are found by multiplying the observations by the fifth and seventh columns.

Then the equations of estimation are (table 16) :

TABLE 16

Equations of estimation

$$\begin{array}{lll} I_{11} & dy_1+I_{12}dy_2 & = S_1 \\ I_{12} & dy_1+I_{22}dy_2 & = S_2 \end{array}$$

of which the calculated values are :

$$\begin{array}{l} 2478{\cdot}5350\ dy_1+\ 179{\cdot}2281\ dy_2 = 14{\cdot}0880 \\ 179{\cdot}2281\ dy_1+3463{\cdot}2909\ dy_2 = 16{\cdot}3989 \end{array}$$

These equations are solved by inverting the information matrix, which then becomes the Covariance matrix (table 17). The value of D is $(I_{11}\times I_{22})-(I_{12}\times I_{12})$.

TABLE 17

Covariance matrix (per cent.)^2

$$\begin{Bmatrix} \frac{I_{22}}{D}=V_{11} & \frac{-I_{12}}{D}=V_{12} \\ \frac{-I_{12}}{D}=V_{12} & \frac{I_{11}}{D}=V_{22} \end{Bmatrix}\times 10{,}000. \quad \text{This is} \quad \begin{Bmatrix} 4{\cdot}049797 & -0{\cdot}209580 \\ -0{\cdot}209580 & 2{\cdot}898273 \end{Bmatrix}$$

V_{11} and V_{22} are the sampling variances of the trial estimates y_1 and y_2 ; dy_1 and dy_2 are the adjustments, and can be calculated as follows :

$$\begin{array}{ll} dy_1 = V_{11}S_1+V_{12}S_2 & dy_2 = V_{22}S_2+V_{12}S_1 \\ \quad = 0{\cdot}530 \text{ per cent.} & \quad = 0{\cdot}446 \text{ per cent.} \end{array}$$

These are added to their respective trial y values to give corrected estimates, and the standard errors are derived from the square roots of the corresponding variances. The map distances (x) are obtained from the Kosambi formula :

$$x = \tfrac{1}{4}\log_e\left(\frac{1+2y}{1-2y}\right)$$

Conversion of y into x can readily be made by interpolation in Table VII, p. 46, of Fisher and Yates (1953), using the identities $y = \frac{1}{2}r$ and $x = \frac{1}{2}z$. The corrected estimate of y_{1+2} is obtained by substituting the corrected y_1 and y_2 values in the expression

$$(y_1+y_2)/(1+4y_1y_2).$$

The fitted map is, then, as in table 18 :

TABLE 18

The fitted map

Adjustments (per cent.)	Corrected estimates (per cent.)	Map distances
$dy_1 = 0{\cdot}530$	$y_1{}^* = 35{\cdot}536 \pm 2{\cdot}012$	44·432 cM (*a*^{t}-*fi*)
$dy_2 = 0{\cdot}446$	$y_2{}^* = 20{\cdot}446 \pm 1{\cdot}702$	21·718 cM (*fi*-*Sd*)
	$y_1{}^*{}_{+2} = 43{\cdot}376$	66·150 cM (*a*^{t}-*Sd*)

A test for the fit of a Kosambi map is given by χ^2 for the goodness of fit of the expectations (m_1) with the observed values in the classes (0), (1), (2), (12) (using $y_1{}^*$ and $y_2{}^*$ in place of y_1 and y_2 in the equations used to obtain m_o), as follows (table 19) :

TABLE 19

Test of Kosambi fit

Exp. m_1	Obs.	(Obs.-Exp.)^2/Exp.
276·7660	271	0·1201
160·7810	166	0·1694
77·7860	82	0·2283
34·6670	31	0·3879
550·0000	550	0·9057 = χ^2 for 1 d.f.

This χ^2 is, as expected, smaller than was obtained in the earlier test (table 14). In both cases it is insignificant.

The value of K is derived from the formula

$$\begin{aligned} K &= (12)\ N^2/2\{(2)+(1)\}\ \{(2)+(12)\}\ \{(1)+(12)\} \\ &= 31 \times 550^2/2 \times 248 \times 197 \times 113 = 0{\cdot}8493, \end{aligned}$$

and its standard error is $1-K/\chi$. These values are shown below (table 20) :

TABLE 20

K and s.e. of K

χ^2 for 1 d.f.	χ	K	1−K	s.e. of K.
0·9057	0·95168	0·8493	0·1507	0·1584

K is thus not significantly different from unity, and a Kosambi degree of interference may be accepted as operating in the segment *a*^{t}*-Sd* in female gametogenesis.

(b) *Male gametogenesis*. The tables for male gametogenesis corresponding to tables 18 and 20 are as follows (tables 21 and 22) :

TABLE 21

Kosambi map for males

Adjustments (per cent.)	Corrected estimates (per cent.)	Map distances
$dy_1 = -0{\cdot}209$	$y_1^* = 26{\cdot}791 \pm 1{\cdot}883$	29·92 cM (*a*^{t}*-fi*)
$dy_2 = -0{\cdot}244$	$y_2^* = 23{\cdot}756 \pm 1{\cdot}814$	25·84 cM (*fi-Sd*)
	$y_{1+2}^* = 40{\cdot}29013$	55·76 cM (*a*^{t}*-Sd*)

TABLE 22

K and its s.e. for males

χ^2 for 1 d.f.	χ	K	1−K	s.e. of K
2·7896	1·6702	0·7113	0·2887	0·1729

(c) *Joint map and K value.* It is useful for making overall comparisons with other chromosomes to have a map (table 23) from the combined data from male and female heterozygotes (trial values

TABLE 23

Combined Kosambi map

Adjustments (per cent.)	Corrected estimates (per cent.)	Map distances
$dy_1 = 0{\cdot}1438$	$y_1^* = 31{\cdot}1438 \pm 1{\cdot}3837$	36·41 cM (*a*^{t}*-fi*)
$dy_2 = 0{\cdot}1067$	$y_2^* = 22{\cdot}1067 \pm 1{\cdot}2457$	23·74 cM (*fi-Sd*)
	$y_{1+2}^* = 41{\cdot}7522$	60·15 cM (*a*^{t}*-Sd*)

taken are $y_1 = 31$ per cent., $y_2 = 22$ per cent., $y_{1+2} = 41{\cdot}6404553$ per cent.).

That the values of K for male and for female gametogenesis are not significantly different can be seen from the following table (table 24), for twice the standard error of the difference appreciably exceeds the difference :

TABLE 24

Insignificance of difference between K values

K s.e.	Difference	Twice s.e. of difference
♀ : $0{\cdot}84930 \pm 0{\cdot}15835$ ♂ : $0{\cdot}71126 \pm 0{\cdot}17288$	$0{\cdot}13804$	$0{\cdot}4688036$

It is thus valid, and will be useful, to calculate the combined value of K (table 25) :

TABLE 25

Joint K and its s.e.

$1/(\text{s.e. of K})^2$ K	Joint K	Variance of K	s.e. of K
♀ $39{\cdot}8808{\cdot}00 \times 0{\cdot}84930 = 33{\cdot}8707634$ ♂ $33{\cdot}458810 \times 0{\cdot}71126 = 23{\cdot}7979132$ $73{\cdot}339610$ $57{\cdot}6686766$	$\frac{57{\cdot}6686766}{73{\cdot}339610} = 0{\cdot}786324$	$1/73{\cdot}339610$	$0{\cdot}116769$

6. DISCUSSION

Owen (1953*a*) obtained for the segment *a-un-we-pa* the following K values :

For females : $0{\cdot}1624 \pm 0{\cdot}1641$
For males : $0{\cdot}9663 \pm 0{\cdot}6829$
Combined : $0{\cdot}2092 \pm 0{\cdot}1509$

The combined value of K is thus significantly different from unity, " being suggestive of a region towards the chromosome terminus or at least between mid-arm and the terminus. The segment *agouti-pallid* is thus between 30-100 cM from the centromere according to what is assumed for the map length of the arm."

The several and combined values of K for the segment a^t-*Sd* are not significantly different from unity. The degree of interference may be said, here, to be on a Kosambi level, in contrast with the smaller portion a^t-*pa*. The fact that the actual values are less than unity militates against the opinion that a^t-*Sd* encloses the centromere or at least that the centromere is only 30 cM from *agouti*—and favours the view that the centromere is beyond *Sd*.

However, the data are not entirely inconsistent with the location of the centromere between *fi* and *Sd*, a position favoured by affinity

studies (Michie, 1953 ; Wallace, 1953), for it is very probable that *a^t-Sd* is nearer the centromere than *a-pa*, and a chromosome arm of 60 cM is the average that may be expected on the basis of chiasmata counts. A brief account of the evidence from affinity studies for the position of the centromere in chromosome V has been given (Wallace, 1954*c* and 1957), and a fuller account will be given shortly with some discussion (Wallace 1958.)

More accurate mapping of the centromere by means other than affinity data may be hoped for from the five-point *Ra-a^t-we-fi-Sd*, which should give a K value based on homogeneous data for each of the overlapping triple segments *Ra-we*, *a^t-fi* and *we-Sd*.

It is not unlikely that the results of this experiment, and of those with other linkage groups now in progress, together with affinity studies will call for, and make possible, some refinements of the current theory of interference.

7. SUMMARY

Interference theory is in advance of experiment. A balanced linkage backcross was undertaken in 1950 and completed in 1952 as a start in supplying the deficiency of reliable experimental material.

Short notes are given on the development of modern interference theory and of the ideas leading to current methods of designing and analysing linkage experiments.

The loci agouti (*A*), fidget (*fi*), and Danforth's short tail (*Sd*) were chosen to demonstrate the use of balance in eliminating disturbance to linkage due to differential viability. Some 1099 progeny were bred.

An analysis of viability relations shows that, despite the low survival rates of fidget and Danforth's short tail (72 and 80 per cent. respectively), there is no appreciable disturbance of linkage either in the data from male or in that from female heterozygotes. There is subsignificant evidence for a kind of position effect apparent as heterogeneity in the survival ratios from the four types of female heterozygote.

Changes in the recombination values in response to both sex and age are examined. Recombination in the segment *A-fi* is significantly higher in females than in males ; in the segment *fi-Sd*, there is a subsignificant difference in the opposite direction. There is a significant increase in recombination in males in the *A-fi* segment with advancing age, but no appreciable change in females.

These observations, when compared with those for the agouti-undulated-wellhaarig-pallid (*A-un-we-pa*) segments of the same chromosome, reveal a rather complex situation. A simple explanation is offered, but further data, of the kind now being produced with five loci in this linkage-group, are needed before its full applicability may be ascertained.

An incidental anomaly is briefly discussed, namely a rise in the sex-ratio with increase in maternal age.

The three-point analysis discloses significant interference. The Kosambi values (K) are: $0{\cdot}7113 \pm 0{\cdot}1729$ for male gametogenesis, and $0{\cdot}8493 \pm 0{\cdot}1584$ for female. As these values are not significantly less than unity, it may be supposed that interference is at a Kosambi level, and a map is fitted accordingly for the separate and combined data. A method of fitting Kosambi maps, based on Fisher's scoring technique, is demonstrated.

A K value of unity may be expected for segments in mid-arm. The three-point values thus agree with the supposition that the whole segment *A-Sd* excludes both centromere and terminus. They do not, however, preclude the possibility, for which other data for the smaller sections of this region provide some support, that the centromere is between fidget and short tail. This hypothesis is favoured by "affinity" studies.

Acknowledgment—It is a pleasure to acknowledge the guidance I have received from Professor Sir Ronald Fisher during the course of this research.

APPENDIX

TABLE I

The frequencies of all genotypes from the three-point backcross

In the description of each genotype, which runs vertically, the symbol a^t stands for the agouti allele used as the dominant and *a* for that used as the recessive: the particular alleles used in each case can be seen from the genotype of the heterozygous parent given in the left-hand margin.

		a^t *Sd*	*fi*	*Sd*	a^t *fi*	*fi* *Sd*	a^t	a^t *fi* *Sd*	*a*	Totals
A. From female heterozygotes										
Tricoupling :	a^t *Sd/a fi*	28	18	13	16	6	8	3	4	96
	A^v *Sd/a fi*	11	8	7	7	1	6	...	...	40
a^t-out :	*a Sd/a^t fi*	19	27	33	36	5	2	10	11	143
Sd-out :	a^t*/a fi Sd*	7	3	3	1	18	28	8	20	88
	A^v*/a fi Sd*	2	1	1	...	7	6	3	4	24
	A/a fi Sd *	...	...	...	1	3	2	1	1	8
	A/a^t fi Sd †	1	1	...	1	4	3	2	2	14
fi-out :	*a^t fi Sd/a*	6	4	12	13	11	25	16	50	137
		74	62	69	75	55	80	43	92	550
B. From male heterozygotes										
Tricoupling :	*a^t Sd/a fi*	19	28	15	6	5	11	3	...	87
	A^v Sd/a fi	10	14	9	6	5	4	...	...	48
a^t-out :	*a Sd/a^t fi*	17	8	42	35	1	6	7	21	137
Sd-out :	*a fi Sd/a^t*	16	8	1	4	30	41	15	24	139
fi-out :	*a^t fi Sd/a*	6	1	14	19	7	20	24	47	138
		68	59	81	70	48	82	49	92	549

* mated to *a^{t}a fifi*. † mated to *a^{t}a fifi* and *aa fifi*.

TABLE 2

Single-factor ratios

	Sd : +	χ^2	*fi* : +	χ^2	a^t : *a*	χ^2
A. From female heterozygotes						
Tricoupling	69 67	0·02941	59 77	2·38235	a^t : *a* 55 41	2·04167
					A^v : *a* 24 16	1·60000
a^t-out	67 76	0·56643	78 65	1·18182	a^t : *a* 67 76	0·56643
Sd-out	60 74	1·46269	54 80	5·04478	a^t : *a* 44 44	0·00000
					A^v : *a* 11 13	0·16667
					A : a^t, *a* 11 11	0·00000
fi-out	45 92	16·12409	44 93	17·52555	a^t : *a* 60 77	2·10949
Total χ^2	241 : 309	18·18262	235 : 315	26·13450	272 : 278	6·48426
Deviation χ^2		8·40727		11·63636		0·06545
		9·77535		14·49814		6·41881
Corrections		1·01155232		1·02161432		1·00011902
Heterogeneity χ^2		9·92709 (3 d.f.)		14·81151 (3 d.f.)		6·41957 (6 d.f.)
Probability		0·02-0·01		0·01-0·001		0·5-0·3
B. From male heterozygotes						
Tricoupling	66 69	0·06667	67 68	0·00741	a^t : *a* 39 48	0·93103
					A^v : *a* 20 28	1·33333
a^t-out	67 70	0·06569	51 86	8·94161	a^t : *a* 65 72	0·35766
Sd-out	62 77	1·61871	57 82	4·49640	a^t : *a* 76 63	1·21583
fi-out	51 87	9·39130	51 87	9·39130	a^t : *a* 69 69	0·00000
Total χ^2	246 : 303	11·14237	226 : 323	22·83672	269 : 280	3·83785
Deviation χ^2		5·91803		17·13843		0·22040
		5·22434		5·69829		3·61745
Corrections		1·01089713		1·03222349		1·00040162
Heterogeneity χ^2		5·28127 (3 d.f.)		5·88191 (3 d.f.)		3·61890 (4 d.f.)
Probability		0·2-0·1		0·2-0·1		0·5-0·3

N.B.—(i) The heterogeneity χ^2 in this and subsequent tables is obtained as follows. It is assumed that each expected two-class ratio is 1 : 1. The χ^2 derived by subtraction of the deviation χ^2 from the total χ^2 is then multiplied by $N^2/4AB$ where A = the total observed number in one class, B = the total observed number in the other, and A+B = N. (In the *Sd* : + series above, A = 246, B = 303, N = 549.) This gives the corrected heterogeneity χ^2. It has the same value as that calculable by taking the observed ratio A : B as the expected.

(ii) The contingency χ^2 values throughout the paper are calculated with Yates' correction for discontinuity.

TABLE 3

Heterogeneity in genotypic ratios within complementary pairs

A. Analysis for three loci simultaneously : Progeny from female heterozygotes (A^y, A, a^t lumped as "a^t" in contrast to "a")

	$\frac{a^t}{fi}$ / Sd	a	χ^2	$\frac{fi}{Sd}$	a^t	χ^2	$\frac{a^t}{fi}$	Sd	χ^2	$\frac{a^t}{Sd}$	fi	χ^2
Tricoupling	3	4	0·1429	7	14	2·3333	23	20	0·2093	39	26	2·6000
a^t-out	10	11	0·0476	5	2	1·2857	36	33	0·1304	19	27	1·3913
Sd-out	14	27	4·1220	32	39	0·6901	3	4	0·1429	10	5	1·6667
fi-out	16	50	17·5152	11	25	5·4444	13	12	0·0400	6	4	0·4000
Total χ^2			21·8277			9·7535			0·5226			6·0580
Deviation χ^2	43	92	17·7852	55	80	4·6296	75	69	0·2500	74	62	1·0588
Corrections			4·0425 1·1517			5·1239 1·0355			1·2726			4·9992
Corrected heterogeneity χ^2 for 3 d.f.			4·6557			5·3058						

Total heterogeneity $\chi^2 = 16·2333$ (12 d.f.).
Probability is 0·30–0·20.

B. Analysis for *fi* and *Sd* loci—a^t locus disregarded : Progeny from male and female heterozygotes

	(i) Female heterozygotes					(ii) Male heterozygotes				
	$\frac{fi}{Sd}$: +		χ^2	fi : Sd		χ^2	$\frac{fi}{Sd}$: +		χ^2	fi : Sd		χ^2
Tricoupling	10	18	2·2857	49	59	0·9259	13	15	0·1429	54	53	0·0093
a^t-out	15	13	0·1429	63	52	1·0522	8	27	10·3143	43	59	2·5098
Sd-out	46	66	3·5714	8	14	1·6364	45	65	3·6364	12	17	0·8621
fi-out	27	75	22·5882	17	18	0·0286	31	67	13·2245	20	20	0·0000
Total χ^2			28·5882			3·6431			27·3181			3·3812
Deviation χ^2	98	172	20·2815	137	143	0·1286	97	174	21·8782	129	149	1·4388
Corrections			8·3067 1·0812			3·5145			5·4399 1·0878			1·9424
Het. χ^2 for 3 d.f.			8·9812						5·9175			
Probabilities			0·05-0·02			0·50-0·30			0·2-0·1			0·7-0·5

The total χ^2 for unequal viability within complementary pairs is the sum of the deviation χ^2 values and is 43·7271 for 4 d.f. Probability is $<0·001$.

(ii) The contingency χ^2 values throughout the paper are calculated with Yates' correction for discontinuity.

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LOCATION OF VACILLANS IN LINKAGE GROUP VIII OF THE HOUSE MOUSE

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VACILLANS is a recessive gene in linkage group VIII that causes a complex syndrome involving muscular co-ordination, behaviour and reflexes, as well as some metabolic abnormality (Sirlin, 1956).

The position of *vacillans* relative to two other genes in the same linkage group, *brown* and *misty*, has now been analysed. Females heterozygous for the three markers, in coupling, were backcrossed to triple recessive males, on a non-agouti (*aa*) background.

TABLE 1

Progeny from matings $\frac{vc\ b\ m}{+\ +\ +}$ ♀ × $\frac{vc\ b\ m}{vc\ b\ m}$ ♂

vc b m	+ + +	*vc b* +	+ + *m*	*vc* + +	+ *b m*	*vc* + *m*	+ *b* +	Total
42	118	5	9	9	12	0	0	195

The results of the matings are shown in table 1. It will be seen that the single-factor segregations are faulty. This, which is due to a mortality in the *vc b m* progeny to be discussed later on, does not interfere with the interpretation that follows. The missing *vc* + *m* and + *b* + progenies make it clear that these are the genotypes that would originate from double cross-overs (there was a single doubtful *vc* + *m* animal that, on the weight of the evidence available, has been tabulated as + + *m*). Therefore, the order of the three genes must be *vc-b-m*.

The female recombination values are: *vc-b*, 10·8±2·2 per cent.; *b-m*, 7·2±1·9 per cent.; *vc-m*, 18·0±2·7 per cent. These values are increased by the depletion in the *vc b m* progeny (see below) representing one parental class of gametes, but, in spite of that, none of them differs significantly from previously reported ones: *vc-b*, 5·5±1·9 per cent. (Sirlin, 1956); *b-m*, 7·1±1·1 per cent. (calculated by the present author from Woolley, 1945); or from their summation: *vc-m*, 12·6±2·2 per cent.

Table 2 gives the number of *brown* mice, scored at birth by the colour of the eye, which died before *misty* and *vacillans* were recognisable. In view of the close linkage between these three genes it seems fair to assume that most of the dead browns were, in fact, also misty and vacillans. Inclusion of the dead mice, with this assumption, gives

108 presumed *vc b m* and 132 presumed + + +, which substantially improves the single-factor segregations in table 1.

Most probably the greater mortality of browns reflects the simultaneous occurrence of *vacillans* in a rather unfavourable maternal environment. In the phenotypes dealt with here any mortality is expected to be due to *vacillans*, rather than to *brown* or *misty*, on account of its constitutional weakness. On this assumption, the data seem to show a dependence of the viability of *vacillans* on parity. In table 2 the ratio of dead browns (probable *vacillans*) to dead blacks is twice as large in first litters as it is in succeeding litters. This was

TABLE 2

Mortality of brown *before age of classification for* misty *and* vacillans

Order and quantity of litters	*Brown*	*Black*	Unclassified	Total
First (10) . . .	30	4	9	43
Second (14) . . .	18	6	2	26
Third (7) . . .	16	4	1	21
Fourth (1) . . .	2	...	1	3
	66	14	13	93

not apparent in larger previous data (Sirlin, 1956), and the discrepancy may be a real one based on the different genetical backgrounds.

Misty and *brown* are located in division 78 and in the distal end of division 79, respectively, of chromosome 18 in Slizynski's pachytene map (Slizynski, 1957). Therefore, *vacillans* should be somewhere in division 80. There is now cytogenetical evidence that the centromere in this chromosome is comprised within divisions 78 to 83 of Slizynski's map (Ford, Carter and Hamerton, 1956).

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TWO DIFFERENT RARE EVENTS OCCURRING SIMULTANEOUSLY IN THE HOUSE MOUSE

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In an inbred line in which Caracul (*Ca*, linkage group VI) had been segregating as a backcross for eight generations, there appeared two males which, from their breeding behaviour, were apparently homozygous for this factor. They were members of the same litter, the first produced by their parents. The latter bred seven Caraculs and four normals in three litters, in good agreement with the expected 1 : 1 ratio. The anomalous males (10/130.370c and 10/130.370d) were given several unrelated does besides their first mates from the inbred line, and bred consistently by all of them as homozygous Caraculs, producing no normals out of over two hundred progeny. A summary of these and other relevant data is given in the table.

There is no possibility of mistaken identity. The inbred line segregates in six factors (*Ca*, belted, *bt*, misty, *m*, brown, *b*, silver, *si*, and Fused, *Fu*), which are not found in this combination elsewhere in this laboratory. The anomalous males' phenotypes and breeding performance were such that they could only have come from this line, and their parents bred as expected for all six factors.

Neither does it seem likely that *Ca* was segregating in an unusual fashion in this line or that any other waving factor was present. The eight previous generations produced severally and in the aggregate ratios of Caracul to normal none of which differed significantly from the expected 1 : 1, and three sibs of the anomalous males, mated to normals, also produced good 1 : 1 ratios.

An explanation which at first appears plausible is that the " non-Caracul " parent of the males in question was in fact Caracul. If so, the mating producing them would have been an intercross for Caracul, and the production of two homozygous males out of eleven offspring would not have been surprising. Certainly the ratio seven Caraculs to four normals is not significantly different from 3 : 1. However, the " non-Caracul " parent was scrupulously examined and no trace of curly hair was found. Unfortunately it died before any specific tests of its genotype could be made. But the records showed that it had been outcrossed to an unrelated non-wavy female in a test for another factor. Three young had been classified for waviness and appeared normal. Five others had been killed at five days old but had not been specifically classified for wavy. However, if they had had wavy whiskers this would probably have been noticed. Thus this mating probably produced no wavies out of eight progeny, a result

which, had the " non-Caracul " actually been a Caracul, could only have occurred by chance with a probability of 1 in 256.

It must therefore be concluded that an unusual genetic situation has occurred.

TABLE

The segregation of Ca *and* bt *from two anomalous males and from their parents*

	Segregation of *Ca* and *bt*					Total segregation (including offspring not classified for *bt*)		
	Ca +	*Ca* *bt*	+ +	+ *bt*	Total	*Ca*	+	Total
Parents of males were $\frac{Ca\ +}{+\ bt}$ ♀ × $\frac{+\ bt}{+\ bt}$ ♂ and gave . .	4	2	...	3	9	7	4	11
Male 10/130.370c *Ca bt m b si Fu* (*Ca* + + + + +)								
×sister *btbt* gave . . .	20	2	...	...	22	31	...	31
×five unrelated non-*bt* females gave	...	...	...	...	...	108	...	108
	20	2	...	...	22	139	...	139
Male 10/130.370d (*Ca* + *m b si* +)								
×related *btbt* gave . .	7	8	...	...	15	44	...	44
×four unrelated non-*bt* females gave	...	...	...	...	...	45	...	45
	7	8	...	...	15	89	...	89

The following explanation appears, at first, to be sufficient :

A mutation may have occurred in the production of chromosome VI in two of the gametes from the non-*Ca* parent. These mutant gametes may have carried either Caracul, or another dominant curling factor, here given the symbol " *K* ", closely linked to *Ca*.

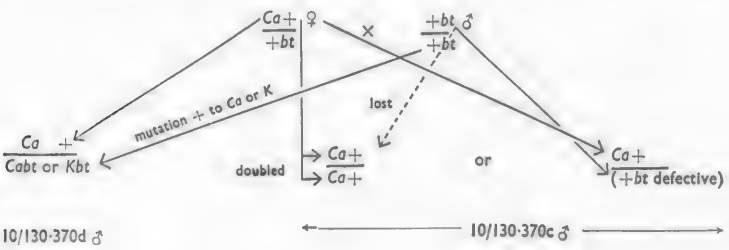
However, on further inspection of the data, it is apparent that a mutational explanation suffices for only one anomalous male's performance. For as regards the *bt* locus, the two males bred very differently (see table). 370d produced eight belted offspring by its related *btbt* mate, thereby demonstrating that it possessed a *bt* chromosome from the *btbt* parent in which the *Ca* or *K* mutation is supposed to have occurred. But its sib 370c, by a similar mate, produced only two belted offspring out of twenty-two classified for this factor, a very poor fit to the half expected. Moreover, the belt of one of these

offspring was poorly expressed and the bearer could be construed as a heterozygote. There is no record of the appearance of the belt of the second offspring, but heterozygous penetrance of *bt* is sufficiently common for one to conjecture that this mouse was a heterozygote also. If, then, it may be said that 370c produced *no* homozygous belteds out of twenty-two, it is not clear what happened to the *bt* chromosome this mouse should have received from its *btbt* father. If a mutational explanation is to be used, one must suppose that two mutations occurred simultaneously in this chromosome, one from normal to *Ca* and one from *bt* to normal.

An explanation for ·370c which requires no mutations at all is as follows.

The 370c zygote may never have received the $+bt$ chromosome from its father, and the $Ca+$ one from its mother may have been doubled ; or it may have lost the $+bt$ chromosome at some time during the early mitotic divisions. Or, if the two apparently belted offspring which 370c produced were actually homozygotes, one may say that the $+bt$ chromosome was not lost, but that it contained some defect which either tended to prevent it from reaching the gametes of 370c or tended to prevent those gametes containing it from reaching the ova of the *btbt* mate. The two *bt* gametes which did fertilise these ova must then have been *Ca bt*, that is, crossovers. (This supposition is consistent with the known *Ca-bt* value for males, 11·09 per cent., Mallyon, 1951). This suggests that the defect was carried in the segment between the *Ca* and *bt* loci. Since the *bt* received by the sib 370d apparently segregated normally, one may suppose that the *btbt* father was at worst heterozygous for the defect. It is conceivable that the same agent was responsible for the mutation to *Ca* or *K* in one chromosome of the *btbt* mouse and for the defect in the other.

To sum up, the anomalous facts are as follows. Two *Ca* males from a coupling backcross of *Ca* and *bt* produced nothing but *Ca* offspring. One male, mated in a backcross, segregated 1 : 1 in normal and belted, while the other, similarly mated, gave only two doubtful belteds out of twenty-two classified. These, and the explanations discussed here, may be graphically represented as follows :



My thanks are due to Professor Sir Ronald Fisher, F.R.S., and to Dr Margaret E. Wallace, for advice.

REFERENCE

MALLYON, S. A. 1951. A pronounced sex difference in recombination values in the sixth chromosome of the house mouse. *Nature*, *168*, 118.

EFFECT ON LINKAGE MAPS OF SELECTION OF CROSS-OVERS BETWEEN CLOSELY LINKED MARKERS

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SEVERAL cases of anomalous frequencies of multiple exchange in small chromosome segments have been reported in the literature. Examples are *Drosophila virilis* (Demerec, 1926), *Neurospora* (Giles, 1951 ; M. Mitchell, 1955 : St Laurence, 1956), *Drosophila melanogaster* (Sturtevant, 1952), maize (Laughnan, 1952), *E. coli* (Rothfels, 1952), *Saccharomyces* (Lindegren, 1953), and *Aspergillus* (Pritchard, 1955). These cases have one feature in common, that is, they show an excess of double exchanges : therefore they have a direct bearing on two of the most important problems of genetics, the linear arrangement of the genetic material and the mechanics of exchange.

Selection for wild-type crossovers from *trans* heterozygotes for two adenineless alleles in *Aspergillus nidulans* was found to be associated with a lengthening of the map in adjacent segments of the chromosome (Pritchard, 1955). The increase was up to about 100 times over the length based on mapping by the analysis of random strands. This same situation was found in another group of adenineless mutants—the object of the present paper—which turned out to be particularly suitable material for the problem under investigation ; namely, the mechanism of crossing over in very short chromosome regions.

1. EXPERIMENTAL

(i) *Materials*

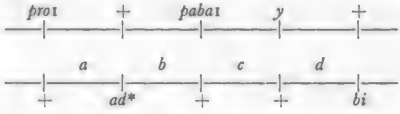
The techniques and the symbols are those described by Pontecorvo (1953). The four adenineless mutants used in these experiments, *ad*9 (S_5D_1), *ad*13 (S_5C_4), *ad*15 (S_5E_4) and *ad*17 (S_5E_6) were obtained by Macdonald and Pontecorvo (1953) by U.V. treatment of a biotin-requiring strain followed by " starvation ". They require adenine hydrochloride at an optimal concentration of 0·09 mg. per ml. of medium ; adenosine and hypoxanthine would also support their growth. The four strains are indistinguishable by means of growth response tests. All are self-sterile as most other adenineless mutants of *A. nidulans*. All crosses two by two are also sterile, except the cross *ad*15 × *ad*17 which gives only crossed perithecia and no selfed. Only these two mutants were extensively used in crosses. Both germinate on media lacking adenine but do not grow any further. The back mutation rate, which was measured in conidia, was very low : only one supposed reversion of *ad*15 was found in about 10^9 conidia tested of each mutant. Self-sterility, cross-fertility, low back

mutation rate and nutritional behaviour made these two mutants (*ad*15, *ad*17) particularly suitable for the present work.

TABLE 1

Localisation of ad15 *and* ad17 *by non-selective analysis*

Assumed order and crossing over intervals



Phenotypes				Crossovers	Crossover frequencies in :			
					cross with *ad*15		cross with *ad*17	
					found	expect.	found	expect.
pro	*ad*	*paba*	*y*					
−	+	−	−	parent 1	490		91	
+	−	+	+	parent 2	436	926	89	180
						919·22		177·84
−	−	+	+	*a*	51		6	
+	+	−	−		68	119	10	16
						114·85		17·78
−	+	+	+	*b*	2		1	
+	−	−	−		1	3	0	1
						3·65		0·714
−	+	−	+	*c*	74		20	
+	−	+	−		80	154	17	37
						147·13		38·64
−	−	−	−	*ab*	1		0	
+	+	+	+		1	2	0	
						0·48		…
−	−	+	−	*ac*	6		4	
+	+	−	+		7	13	2	6
						18·27		3·86
−	+	+	−	*bc*	0		0	
+	−	−	+		1	1	0	
						0·60		…
−	−	−	+	*abc*	0		0	
+	+	+	−		0		0	
						0·07		…
					1218		240	

* Stands for *ad*15 or *ad*17 according to the cross.

(ii) *Experiments*

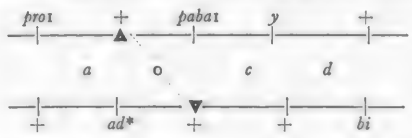
The mutants *ad*15 and *ad*17 were known to be related to *ad*9 which is very closely linked to *paba*1 ; heterokaryons *ad*9 + *ad*15 and *ad*9 + *ad*17 were adenine requirers, suggesting allelism (Pritchard, unpublished).

Equally, the heterokaryon *ad*9 + *ad*13 turned out to be adenine-requirer. These crude tests suggested that all four mutants might belong to one cluster of alleles.

Starting from this information *ad*15 and *ad*17 were localised by crossing the original strains *ad*15 *bi* and *ad*17 *bi* with the strain *pro*1 *paba*1 *y*. A random sample of crossed spores was first examined

TABLE 2

Localisation of ad15 *and* ad17 *by selective analysis*
(*selection for* ad+ paba1+)



The dotted line shows the selected crossovers (indicated as o)

Phenotypes			Crossovers	Crossover frequencies in	
				cross with *ad*15	cross with *ad*17
pro	*y*	*bi*			
−	+	−	o	51	144
+	+	−	*a*	17	19
−	−	+	*c*	19	47
−	+	+	*d*	5	11
+	−	+	*ac*	5	6
+	+	+	*ad*	5	4
−	−	−	*cd*	3	2
				105	233

Classification for mapping of the segment *paba*1 *bi*

Phenotypes		Crossovers	Found	Expect.	Found	Expect.
y	*bi*					
+	−	o	68	68·34	163	164·0
−	+	*c*	24	23·66	53	50·7
+	+	*d*	10	9·66	15	17·0
−	−	*cd*	3	3·34	2	3·9
			105		233	

* Stands for *ad*15 or *ad*17 according to the cross.
▲▼ Indicates the points between which crossing over is selected.

with the results shown in table 1. In cross *ad*15 *bi* × *pro*1 *paba*1 *y* out of 1218 ascospores examined there were 6 recombinants in the interval *ad-paba*. Three of them represented also a further exchange either in region *a* or region *c*. In cross *ad*17 *bi* × *pro paba y* only one crossover in *b* was found out of 240 ascospores. These data gave a first indication of the location of the mutants : both *ad*15 and *ad*17 are closely linked to *paba* and most likely between *pro and paba*.

Decisive evidence came from selecting recombinants *ad*+ *paba*1+ (independent of both adenine and *p*-aminobenzoic acid) from the same pools of ascospores used in the non-selective platings of table 1. The data are reported in table 2. Unmistakably the sequence is :

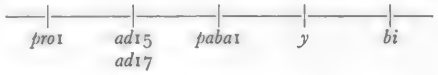


TABLE 3

Estimation of the recombination fraction between paba1 *and* ad15 *or* ad17

Colonies on complete medium		Colonies on minimal medium supplemented with proline and biotin	
Cross involving		Cross involving	
*ad*15	*ad*17	*ad*15	*ad*17
No./plate	No./plate	No./plate	No./plate
41	46	8	11
34	48	8	4
44	65	9	9
21	46	6	17
33	43	10	11
28	45	7	5
34	66	7	6
31	64	10	12
34	42	4	3
17	64	3	7
317	529	72	85

Platings of ascospores from a single perithecium of crosses :

*ad*17 (or *ad*15) *bi*×*pro*1 *paba*1 *y*

The same suspension of ascospores from each cross was plated undiluted on selective medium, diluted 1/60 (a separate dilution for each plate) on complete medium. The double of the ratio of mean number of colonies on selective medium to that on complete medium (×60) is an estimate of the recombination fraction between *ad*15 (or *ad*17) and *paba*1.

Using new pools of ascospores from crossed perithecia of the same crosses, the best estimate of the recombination value between *ad* and *paba* was calculated from the ratio of recombinants requiring neither adenine nor *p*-aminobenzoic acid to the total of viable ascospores. This ratio was obtained from plating two parts of a single suspension : plating (1) on selective medium (devoid of *p*-aminobenzoic acid and adenine), ten separate samples ; plating (2) on complete medium, ten separately diluted samples. The dilution in plating (2) was 1/60 (table 3). The recombination values calculated from these data are

$3{\cdot}3\times10^{-3}$ for the interval *ad*15-*paba*1 and $2{\cdot}4\times10^{-3}$ for the interval *ad*17-*paba*1. The difference between these values is not significant.

Similar crosses involving *ad*9 and *ad*13 located also these two mutants at practically the same position as *ad*15 and *ad*17. Full details are not given here because they are irrelevant for the present paper.

Recombination between ad15 *and ad*17. The experimental evidence so far was still compatible with the possibility that *ad*15 and *ad*17 might represent repeated isolation of the same mutant. However, as mentioned before, crosses between *ad*15 and *ad*17 were fertile: showing that the two mutants were complementary (in respect of fertility), *i.e.* not identical at least functionally. As an extrapolation, it was expected that *ad*15 and *ad*17 represent mutations at two different sites (Pontecorvo, 1952), distinguishable by means of crossing over. To test this expectation, two groups of crosses were made, differing from one another in that the two adenine mutants were in reciprocal positions in respect of the other markers used. From the two groups, only the following two crosses will be reported in full detail here because though all of them gave concordant results only these two were planned for a comparison. These crosses are:

Cross A

	a	*b*	*c*	*d*	*e*	
Parent 1	+	*ad*17	+	*paba*1	*y*	+ *w*$_n$
Parent 2	*pro*1	+	*ad*15	+	+	*bi* +

Cross B

	a	*b*	*c*	*d*	*e*	
Parent 1	+	+	*ad*15	*paba*1	*y*	+ *w*$_n$
Parent 2	*pro*1	*ad*17	+	+	+	*bi* +

Large numbers of ascospores could be readily collected from these crosses. That all these spores were of crossed origin was shown by testing about 50 perithecia from each of cross A and B. These perithecia were picked at random and separately streaked on complete medium; they invariably gave rise to three-colour streaks (green, white and yellow). Green and white are parental colours; yellow is recombinant (Hemmons, Pontecorvo and Bufton, 1953). Both from crosses A and B large numbers of spores were collected free from conidial contamination. Samples of these spores were plated on minimal medium supplemented with proline, *p*-aminobenzoic acid and biotin but not with adenine, and on complete medium. On complete medium the viable counts were 100 per cent. in almost every case. On the medium without adenine only adenine-independent colonies grew, together with a few poorly growing heterokaryons and a few diploids. Heterokaryons and diploids are easily recognised (Pritchard, 1954) and have been removed from the analysis.

The frequency of real adenine-independent colonies was about 3×10^{-6}.

The results of the selective platings are shown in table 4. The majority (57 per cent. in cross A, 59·5 per cent. in cross B) of the

TABLE 4

Segregation frequencies in crosses A and B among selected adenine-independent recombinants

Cross A

	a	*b* ▼	*c*	*d*	*e*	
Parent 1	*ad*17		*paba*1	*y*	+	w_n
Parent 2	*pro*1	*ad*15	+	+	*bi*	+

▲

Cross B

	a ▼	*b*	*c*	*d*	*e*	
Parent 1	+	*ad*15	*paba*1	*y*	+	w_n
Parent 2	*pro*1	*ad*17	+	+	*bi*	+

▲

Classification of recombinants in respect of *pro*1 and *paba*1

Phenotypes		Cross A	Cross B
pro	*paba*		
−	+	57	19
+	−	26	46
+	+	10	166
−	−	123	7

Classification for mapping of the segment *paba-bi*

Crossovers	Cross A					Cross B				
	paba	*y*	*bi*	Found	Exp.	*paba*	*y*	*bi*	Found	Exp.
0	−	−	−	120	119·21	+	+	−	92	92·59
c	+	−	−	51	53·58	+	−	+	41	39·24
d	−	+	−	21	21·47	−	−	−	19	21·85
e	−	−	+	8	6·91	+	+	+	10	8·50
cd	+	−	−	12	9·63	−	+	−	11	9·25
ce	+	−	+	4	3·13	+	+	−	1	3·59
de	−	+	+	0	1·25	−	−	+	4	1·99
cde	+	+	+	0	0·56	−	−	+	0	0·83
				216	215·74				178	177·84

▲▼ Indicates the points between which crossing over is selected.

adenine-independent colonies are recombinant between *pro*1 and *paba*1 ; in both crosses A and B the spectrum is in accord with the hypothesis of two sites of mutation and the sequence is the following :

*pro*1 — *ad*17 — *ad*15 — *paba*1

Keeping in mind that the two spectra are reciprocal, the results of the selective platings from the two crosses are homogeneous, *i.e.* viability effects could easily account for the minor differences observed which incidentally are not statistically significant. By pooling the data the following map is obtained :

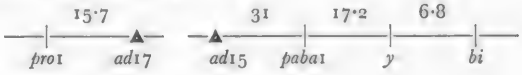


TABLE 5

Segregation frequencies in crosses A and B among non-selected ascospores

Phenotypes				Crossovers	Cross A		Cross B	
					Found	Expect.	Found	Expect.
pro	*paba*	*y*	*bi*					
+	−	−	+	parent 1	65	140	131	206
−	+	+	−	parent 2	75		75	
+	+	+	−	*a*	4	8	8	12
−	−	−	+		4		4	
+	−	+	−	*d*	4	6	10	17
−	+	−	+		2		7	
+	−	−	−	*e*	1	2	4	10
−	+	+	+		1		6	
+	+	−	+	*ad*	2	3	1	7
−	−	+	−		1		6	
+	−	+	+	*ae*	0	1	1	2
−	−	−	−		1		1	
+	−	+	+	*de*	0	…	0	1·02
−	+	−	−		0	…	2	2 …
+	+	−	−	*ade*	0	…	0	…
−	−	+	+		0	…	0	…
					160		256	

One-half of the recombinants in crosses A and B carried w_n (white) epistatic to *y* (yellow) and *y*+ (green). One-third of the whites were outcrossed to ascertain the presence of *y* or *y*+ and estimate it for the rest.

This map will have to be compared with one based on the results of non-selective platings from the same crosses. The data are in table 5.

In cross B the frequencies of the complementary crossover classes are heterogeneous (see for instance the parental classes, one of which is almost double the other). This heterogeneity is probably due to lower viability of prolineless spores which is known to occur on some batches of complete medium. (The possibility of contamination by selfed parental spores or by conidia has been ruled out.) Low viability of *pro*1 would affect the formal analysis by increasing the crossover values of *a* and *e* and decreasing the coincidence value.

In the relatively small samples of spores analysed in non-selective platings no recombination between *ad*15 and *ad*17 is expected, nor was it observed : the crosses behave as if homozygous *ad*15 or *ad*17 and can not yield information as to recombination in region *c* (the segment of chromosome between *ad*15 and *paba*1). However, these

TABLE 6

Comparison between maps based on selective analysis and those based on non-selective analysis in crosses heterozygous ad15/ad17

	Cross A		χ^2	Cross B		χ^2	Cross A′		χ^2	Cross A″		χ^2
	Selective	Non-selective		Selective	Non-selective		Selective	Non-selective		Selective	Non-selective	
Centromere ●												
*pro*1												
a	16·6	7·5	6·98	14·6	8·2	4·5	...	...	...	29·6	8·9	18·1
*ad*17 *ad*15 ◀▶												
c	31·0	(0·3)*	...	29·0	(0·3)*	...	38·8	(0·3)*	...	32·4	(0·3)*	...
*paba*1												
d	15·0	5·6	27·0	19·0	10·15	7·05	24·0	11·7	12·7	22·2	15·55	3·2
yellow												
e	5·0	1·8	3·25	8·4	5·4	1·45	9·8	3·14	6·28	13·6	4·0	16·6
bi												
Total number of colonies tested	216	160	...	178	256	...	258	128	...	154	360	...

▲▼ Indicates the points between which crossing over is selected.
* The estimate in brackets derives from the experiment reported in table 3.

Cross A′ :

+	*ad*17	+ ▼	*paba*1	*y*	+
+	+ ▲	*ad*15	+	+	*bi*

Cross A″ :

+	*ad*17	+ ▼	*paba*1	*y*	+
*pro*1	+ ▲	*ad*15	+	+	*bi*

data are valuable because they allow a comparison between selective and non-selective analysis within each cross. This comparison is shown in table 6, on which the results of other similar crosses (A′ and A″) are also included.

The selective analysis (based on data involving a selected crossover between the two *ad*) gives a map-length for region *c* some 30 units greater than that found as the average value in non-selective mapping (see table 1). Again, for region *a* selection gives a map-length some 7 units greater than that found without selection on the same sample of ascospores. The two segments *a* and *c* are both adjacent to the point at which the crossover leading to adenine independence was

TABLE 7

Comparison between maps based on selective analysis and those based on non-selective analysis in crosses pro1 paba1 y×ad15 bi *and* pro1 paba1 y×ad17 bi (*from the data of tables 1 and 2*)

		Cross with *ad*15		χ^2	Cross with *ad*17		χ^2
		Selective	Non-selective		Selective	Non-selective	
Centromere ●							
pro1							
	a	25·7	11·0	20·3	12·4	9·1	1·32
*ad** ▶							
paba1 ◀							
	c	25·7	13·79	15·18	23·6	17·9	1·985
y							
	d	12·3	3·62	11·5	7·3	5·8	0·2
bi							

▲▼ Indicates the points between which crossing over is selected.
* Stands for *ad*15 or *ad*17 according to the cross.

selected. Segments *d* and *e* which are farther away from the point of selection also show an increase in map-length though to a slighter extent.

That the increase in map-length is not a peculiar feature of selection of crossovers between *ad*15 and *ad*17, is shown by two further types of evidence. First, the data in tables 1 and 2, compared in table 7, show that selection for $ad^+ paba^+$ crossovers, *i.e.* between two genes functionally quite distinct and less closely linked (recombination fraction of 3×10^{-3}), determines a small but significant increase of the map-length in both adjacent intervals.

Second, another experiment involving two complementary and closely linked prolineless mutants, *pro*1 and *pro*3 (Forbes, 1956) gave the same sort of results. The experimental procedure was the same as that in crosses A or B, and the results are in table 8.

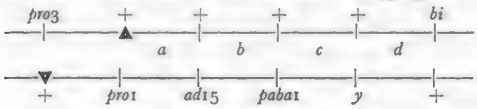
2. DISCUSSION

(i) *Origin of "prototrophic" colonies*

Before discussing the experiments reported here it is necessary to be clear on what gives origin to the non-requiring colonies from

TABLE 8

Comparison between selective and non-selective mapping in cross



Centromere ● — pro3 ▶ — pro1 ◀	Selective	Non-selective	χ^2
a	14·18	8·64	2·86
*ad*15			
b	0·5	0·5	...
*paba*1			
c	16·2	13·7	0·21
y			
d	4·7	4·07	0·09
bi			
Total number of colonies tested	191	196	...

▼▲ Indicates the points between which crossing over is selected.

crosses *ad*17 × *ad*15 and *pro*3 × *pro*1. Discussion of the mechanism leading to their formation is pertinent because the explanation based on crossing over between two sites of mutation might be incorrect since it implies an increased frequency of exchange near the selected locus.

This point has been discussed by Pritchard (1955) when he first found association of prototroph formation with increased recombination near the locus involved. His conclusions based on mitotic analysis —which favour crossing over—are very convincing.

In the present case the selection of adenine-independent recombinants in pairs of crosses with the reciprocal arrangements of markers (crosses A and B) gives spectra of segregation with mirror image patterns (see data in table 4). Again crossing over seems to be the most economical explanation.

Further evidence in favour of crossing over derives from Forbes' (1956) work on prolineless mutants, which Strickland (unpublished) was able to supplement by tetrad analysis. All the asci containing proline-independent recombinants also contained the complementary double mutant recombinants, besides the non-crossover strands.

(II) *The lengthening of the maps in selective analysis*

Two groups of models have been put forward to explain cases of anomalous frequencies of what appear as products of multiple exchanges within small chromosome regions. First, those which suggest special mechanical devices of recombination either based on crossing over or other unknown mechanisms. Second, those which suggest formal kinds of explanation in which a coincidence value higher than one would result because of the inclusion in the calculation of meiotic products in which crossing over was absent.

In the former group one may put Winkler's (1930) "gene conversion", the model of copying choice of Lederberg (1954), and the explanation suggested by Pritchard (1955). The latter group includes those by Rothfels (1952), Sturtevant (1954), Cavalli and Jinks (1954 and 1956). While the first group might have an advantage in the possibility of providing also a model for the duplicative process of the chromosome, the second is more in keeping with the well-established production of complementary strands in crossing over. These two categories are not necessarily conflicting; in fact the final explanation valid for all the data that are being collected might turn out to be an integrated form of both of them.

In *A. nidulans* Pritchard (1955) found a great increase, compared to the expectation based on mapping by random strand analysis, in the number of exchanges in a segment of chromosome next to one within which a crossover between adenineless alleles was selected. A further investigation by Forbes (1956) on two other groups of alleles (*pro*1 and *pro*3) gave the same pattern of recombination. The present investigation yields results strictly similar to those of Pritchard; it shows an increase in map length with the following features: (*a*) the effect spreads beyond the chromosome segments adjacent to the point of selection (table 6 compares maps from selective and from non-selective analysis and shows the increases in map-length in non-adjacent regions and the falling-off of this effect with the distance from the point of selection); (*b*) the magnitude of increase appears to be related to the closeness of linkage between the markers used for selection. Table 6, where selection was for *ad*15$^+$ *ad*17$^+$, shows the effect of a selection 1000 times stronger than table 7, where selection was for *ad*15$^+$ *paba*1$^+$, or table 8, where selection was for *pro*3$^+$ *pro*1$^+$.

A tentative model for a theory of incomplete pairing offers an interpretation of the experimental data.

I assume that pairing,* as a necessary condition for crossing over, is not complete. In any one population of cells in meiosis for any one chromosome section there is a fraction with no pairing. As a necessary first approximation it is assumed that the chromosome segments which are paired have a random distribution along the chromosome length.

The main consequences of the theory are that the increase in map-length due to selection is the higher the more stringent is the selection, and that this increase becomes smaller with distance from the point of selection.

Let l be the length of the pairing segment, x the length of the segment within which crossovers are selected, a and c the two chromosome segments on each side of x. The frequency of recombination in c in all the zygotes in which a crossover in x is selected will be :

$$f_c = \tfrac{1}{2} \frac{l}{l+x} \quad . \quad . \quad . \quad . \quad . \quad (1)$$

(The factor $\frac{1}{2}$ is taken for a case of maximum frequency of recombination in l ; it will be less than this if the incidence of crossing over in the paired segment is not very high.)

If l (the length of the paired segment) were constant from cell to cell, though its position varied at random over the whole chromosome length, then (1) would give the expected relationship between f_c, an observable quantity, and x a quantity that can be varied by choosing for selection markers varying in their closeness. The curve thus obtained will be of the form given in fig. 1 for $l = 1$, where x represents a multiple of l. It will be seen that for the experiment in which selection is for crossovers between *ad*17 and *ad*15—which are at a distance of 3×10^{-6}—the observed recombination (0·30) in the segment *ad*15-*paba*1 corresponds to a value of $x = 0{\cdot}78$, *i.e.* the length of the pairing segment would be 1·4 times the length of the segment in which the crossover was selected, *i.e.* 0·00038 map units.

If, on the other hand, l were variable from cell to cell, even assuming an extreme case of a skew distribution of the form :

$$d\phi(l) = ke^{-kl}dl$$

the shape of the curve would not vary substantially (fig. 1.), though its values would be somewhat lower. In this case the observed recombination in the segment *ad*15-*paba*1 would correspond to a value of $x = 0{\cdot}35$, and the pairing segment would be about 0·001 map units.

The applicability of this theory rests on the symmetry of the increases on both sides of the point of selection. Let us, thus, consider the increase in segment a (between *pro*1 and *ad*17). The value of recombination in a (p_s) after selection should be :

$$p_s = p_r + p_x - p_r \times p_x$$

* The term " pairing ", as used here, does not assume any relation to cytologically visible pairing as observed in prophase of meiosis. The term is unfortunate but it was used first by Rothfels (1952) and later by Cavalli and Jinks (1954, 1956) with the same meaning as here. Pritchard (1955) used the less confusing expression " effective pairing ".

where p_r is the recombination fraction in non-selective analysis and p_x the expected increase. On the basis of the values in table 6, the expected value works out to be $p_s = 0{\cdot}356$, while, in fact, the found mean value is less than 0·16.

This model was built on the assumption that the pairing segments are distributed at random. The data clearly do not fit this simple form of the model. The theory of incomplete pairing stands on the basis of the qualitative results but the two first approximations, randomness of location and of size of the paired segments, are untenable.

This model concerns only segments adjacent to the point of selection. A more complicated one would be necessary to evaluate

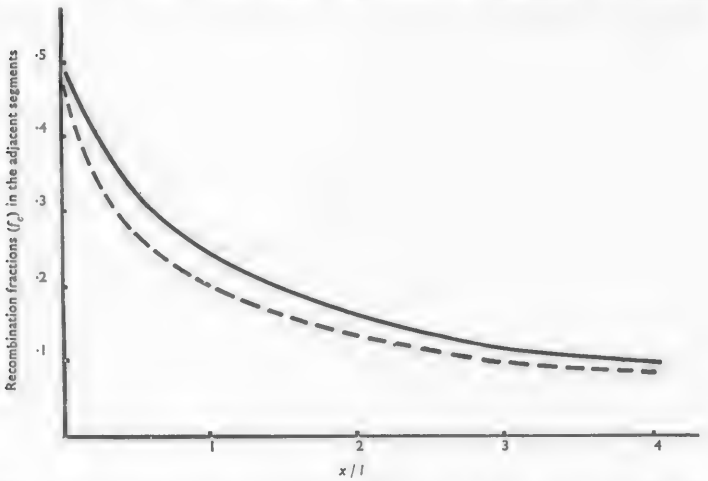


FIG. 1.—Relationship between recombination fractions in an adjacent segment and length x of the segment where crossovers are selected. x is expressed as a multiple of l the length of the pairing segment. The two curves are for constant and highly variable length of the pairing segment, respectively.

the expected *lengthening in non-adjacent segments*. At the moment I will confine the discussion to its qualitative aspects.

The data, though limited, are sufficient to show that lengthening of non-adjacent segments occurs when the strength of selection is of the order required in selection of *ad*17$^+$ *ad*15$^+$ (as in crosses A and B). The problem now is whether this lengthening has the same cause as that in the adjacent segments. In the light of the theory of incomplete pairing, this would mean that at least some of the pairing segments are long enough to cover the portion of chromosome from the point of selection to the last but one marker, a length of 15 units on the non-selective map and 45 units on the selective ones.

As an alternative explanation, let us suppose that the increased recombination in non-adjacent segments were due to selection for

products of cells in meiosis with different proneness to crossing over. Paraphrasing Sturtevant's example (1954, p. 238), suppose we were analysing a mixed population of gametes from males and females of *Drosophila*. The selective analysis would discard the male gametes, and *all* the map-lengths would be increased by a constant factor dependent on the proportion of non-crossover gametes, but independent of the distance from the selected point. The results reported in table 8 seem to exclude this alternative. Here, in fact, the segment adjacent to the point of selection is substantially increased, in selective analysis, while the three segments beyond it are not. The explanation in terms of the theory of incomplete pairing seems to be the simplest. Due to the relatively small strength of selection in this case, the longer paired segments are not selectively picked up as much as in experiments like those of selection for *ad*15$^+$ *ad*17$^+$.

Finally there is evidence in favour of incomplete pairing which is complementary to that discussed so far. That is, coincidence values greater than 1 are found in non-selective analysis. This is the necessary counterpart, expected on theory, of the lengthening of the maps in selective analysis. None of the previous work on *Aspergillus nidulans* had detected any kind of interference because it dealt with regions too large to provide a sensitive test.

The evidence is as follows. The experiment on localisation of *ad*15 reported in table 1 includes two adjacent segments (*a* and *b*) of total length less than ten units. The coincidence value found there is about 4. In crosses A and B the non-selective analysis again gives coincidence values in excess of 1 over total lengths of about 13 units. Although only few recombinants are available for these estimations, the tendency is clear and highly suggestive.

3. SUMMARY

1. The formation of rare prototrophs among ascospores from crosses between two adenineless mutants (*ad*15 and *ad*17) of *Aspergillus nidulans* is most probably due to the occurrence of crossing over between them. Reciprocal crosses yield strictly complementary segregation patterns.

2. Selection for crossovers between very closely linked markers lengthens the linkage maps, as compared with the maps based on non-selective analysis.

3. The increase in recombination frequencies has its maximum near the point of selection. It spreads over segments of chromosome beyond those adjacent to the point of selection. And it is the greater the closer the linkage between the markers used for selection.

4. Parallel to the increase of recombination frequencies in selective analysis, coincidence values higher than 1 are found in non-selective analysis between pairs of segments within an overall length of about 10 units.

5. These increases can be accounted for by assuming that pairing is not complete along the whole length of every bivalent in every cell in meiosis, but partial and variable in length and position. Selection for crossovers at one point would also select the products of those cells in meiosis in which the probability of pairing around the selected point is greater than average.

Acknowledgments.—This work, which is part of a general programme supported by the Nuffield Foundation, was carried out under the tenure of a Nuffield Research Scholarship. The author is deeply indebted for advice and criticism to Professors G. Pontecorvo and L. L. Cavalli-Sforza.

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REVIEW

STATISTICAL METHODS AND SCIENTIFIC INFERENCE. By Sir Ronald A. Fisher. Oliver and Boyd. 1956. Pp. viii+175. 16s.

Almost all the great advances that have been made in statistical method over the last forty years have as their bases ideas developed by Professor Fisher. In two books in this field (1, 2) he laid the foundations of statistical science as we know it to-day. The arrival, therefore, of a third book by the same author is an event of considerable importance: for it will be recognised that, if it is on a par with the other two, its influence on statistical development will be tremendous. Any reviewer is hence faced with the task of determining whether this parity is attained.

The two earlier books were concerned with the practical problems of the design and analysis of experiments: the emphasis was on applications and the bases of the reasoning processes employed were not completely investigated, and have certainly not been completely understood by other workers. The present book has for its purpose "An examination of the logical nature of uncertain inference". In it an attempt is made to define more strictly the concept of mathematical probability and its use in scientific inference. As such the book must be wholly welcome, for it is clear that statisticians to-day are much happier in designing or analysing experiments than they are in explaining just why they are doing what they do. For example, new significance tests are continually being produced but the concept of a significance level is not clearly understood.

The book begins with two chapters which are mainly historical in character. Earlier definitions of probability are considered and a new one proposed. The original feature of the definition is the incorporation into it of a statement of ignorance "specified by our inability to discriminate any of the different sub-aggregates having different limiting frequency ratios" (p. 33). This is obviously related to the view that all probabilities are conditional. A valuable statement is given on p. 35 wherein the author distinguishes three types of uncertain inferences: (i) those involving Bayes' theorem, (ii) tests of significance, (iii) estimation problems. The last two depend on the absence of knowledge *a priori*. The third chapter discusses significance tests, the fiducial argument and the distinction between probability and likelihood. It concludes with an interesting argument involving the likelihood, to be used when true probability statements are not available. The fourth chapter is concerned essentially with the problem of what space we should integrate over in forming our significance levels or fiducial probabilities. The fifth chapter gives further examples of the fiducial argument, including some dealing with the fiducial prediction of future observations. The sixth and final chapter is concerned with estimation. A new and interesting definition of consistency is put forward, a new proof of the asymptotic efficiency property for maximum likelihood estimators is given and the use of ancillary information exemplified in a very pretty example (p. 163 onwards).

In commenting on the book it is convenient to begin with one criticism which is mathematical, and demonstrates that an error has been made,

and to continue with other criticisms which are more matters of opinion. Perhaps most statisticians who are interested in the foundations of their subject will, on seeing the book for the first time, turn its pages to see what the author has to say about fiducial probability. For of all the concepts introduced by him this one has been least understood and been subject to the most criticism. On page 51 there is a definitive footnote which gives some important information :

" Probability statements derived by arguments of the fiducial type have often been called statements of " fiducial probability ". This usage is a convenient one, so long as it is recognized that the concept of probability involved is entirely identical with the classical probability of the early writers, such as Bayes. It is only the mode of derivation which was unknown to them ".

The remark is important because it tells us that we can manipulate fiducial probabilities in the same way as other probabilities, and, in particular, we may expect them to obey Bayes' own theorem. This expectation is supported by the appearance on pages 123-127 of arguments in which fiducial probabilities are used in Bayes' theorem. " This fiducial distribution supplies information of exactly the same sort as would a distribution given *a priori* ". (p. 125). It therefore seems that fiducial probabilities obey Bayes' theorem. However, it is quite easy to demonstrate that they do not. Suppose that from a first sample we obtain a fiducial distribution and use this as a prior distribution in Bayes' theorem for a second, independent, sample. Then if the fiducial argument has the properties that the author claims for it, and if it is to be a consistent mode of reasoning, the posterior distribution obtained by this method should be equal to the fiducial distribution for the combined sample. A counter-example is obtained by taking samples of size one from the distribution with frequency function $\{\theta^2/(\theta+1)\}(x+1)e^{-x\theta}$ for $x \geqslant 0$, $\theta > 0$. It may be proved that in the case of one parameter where a single sufficient statistic exists for samples of any size the fiducial argument is only consistent if the parameter is transformable to one of location. This implies that only in families of normal distributions with known variance, or of gamma distributions with known index, or families which may be transformed thereto, is it possible to apply the fiducial argument. This limitation restricts the usefulness of the idea and makes some results in the book false or without any logical basis.

It is not so clear how such an error came to be made. One possible explanation, which leads to criticisms which are more matters of opinion, is that the error is caused by the author's arguments being almost entirely through the medium of examples. Thus (p. 106) " it is not the mathematics but the logical nature of these concepts that requires to be *exemplified* " (my italics). There can be no objection to examples as such, but without extracting from them those features which are common and relevant and distinguishing these from features peculiar to the examples, it is not possible to know what the author intends. The situations considered in this book are so special, being confined to a restricted set of distributions, that it is not possible to distinguish the particular from the general. As an instance of this consider the ingenious attempt made at the beginning of chapter three to present an argument for significance tests which avoids overt mention

of alternative hypotheses. The example concerns the random distribution of stars over the celestial sphere. Now the general significance test argument uses an integration over the sample space, but in the example the integral is effectively the ordinate at a single point, that is the probability of the observed sample. "The probabilities of having 6 or more close neighbours adding very little to this frequency". (p. 39). The quotation is the only reference to the integration in the example. The author then argues that the basis of a significance test is that "*Either* an exceptionally rare chance has occurred, *or* the theory of random distribution is not true". (p. 39). But in general, and even in other examples in the book, it is not the chance of the event that has occurred that is considered but the chance of the observed event *and other more extreme ones*. Typically the tail of the distribution is used. The introduction of the tail area needs a justification which is not provided by the author: the most usual one is due to Neyman and Pearson and uses the concept of an alternative hypothesis (3). Notice that the casual reader will be led astray, through the use of the particular example wherein a special situation obtains, to thinking that the argument, which carries conviction there, extends easily. This is not so.

A second example follows concerning the significance test for linkage. Here the hypothesis tested is that $\theta = \frac{1}{4}$, representing no linkage. Although the author puts it in the second of his three categories a good case could surely be made for saying that it belongs to the first, because some prior knowledge is certainly available; namely that the genes are linked if, and only if, they lie on the same chromosome. If the species under observation is known to have n chromosomes and if nothing is known about the genes' positions then a prior probability of n^{-1} for linkage immediately suggests itself: that is a prior probability for $\theta = \frac{1}{4}$ of $(n-1)/n$, a rather substantial amount. Any analysis which ignores such a concentration would appear to be ignoring a salient feature of our knowledge of genetical structure.

The distinction between statements of mathematical probability and statements of likelihood is emphasised in several places throughout the book, indeed the first person to point out the important distinction was the author. Nevertheless the practical circumstances under which the two types of argument should be used are not distinguished adequately. For example, in chapter three, the fiducial argument (using probability statements) is given for continuous variables, but when the variables become discrete, in a later example, a likelihood argument is used. It is not clear why a slight change from continuity to discreteness should result in a considerable change in the type of argument. For, in general, a probability, involving an integration over the sample space, will behave in an entirely different manner from the corresponding likelihood which does not use such an integration. What is lacking, for example in the significance test argument cited already, is an adequate justification for the use of the sample space in inference. As Barnard has put it "probabilistic considerations are relevant only before an experiment is performed . . . once the experiment has been performed, our arguments concerning the possible hypotheses which might account for them are to be couched in terms of likelihood, not in terms of probability". (11, pp. 58-59). Similarly Jeffreys has remarked "The use of the P integral in significance tests . . . is

fallacious because it rejects a hypothesis on account of observations that have not occurred " (6, p. 360).

Another criticism of the book is the lack of adequate reference to contemporary writers, and apparent ignorance of their work. For example there is a derivation of the Fisherian significance test for 2×2 tables using Neyman-Pearson principles by Tocher (4), of which the author seems unaware. Another derivation using decision theory ideas is due to Sverdrup (5) and a third, using a Bayesian argument which produces the same criterion though a rather different use of it, is due to Jeffreys (6, §5.11). There is no discussion of the criticism so clearly expounded by Jeffreys (6), one of which has already been cited. Again, the Problem of the Nile, known to other writers as the problem of similar regions, has been solved by Neyman (7) in the case where there are only a finite number of hypotheses (or the Nile can rise to only a finite number of levels). No mention is made of the important inequality due to Cramer (8, p. 480) and others, which is valid for small samples. It is a pity that no discussion of the difficulties raised by Williams (9) or, later, Mauldon (10) in connection with the fiducial argument is included.

This new book is a disappointment. Had it done for the foundations of the subject what the earlier books of the author did for the practical side, statistical science could have progressed still further. As it is, the best that we can hope for is that the book will make statisticians realise the unsatisfactory nature of the Fisherian argument and make them more ready to read and accept the Bayesian argument (6). The worst that can happen is that it should command an important position just because of brilliant work done by the author thirty years ago. D. V. LINDLEY.

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GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND TWENTY-THIRD MEETING of the Society, held on 2nd and 3rd APRIL 1957, in the DEPARTMENT OF GENETICS, THE UNIVERSITY, SHEFFIELD

EFFECTS OF STABILISING AND DISRUPTIVE SELECTION

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Stabilising selection may be defined as selection that favours the mean value for a particular quantitative character. Disruptive selection may be defined as selection that favours *both* extreme values of a character in a single interbreeding population. Each may be expected to have two types of effect. They may so affect the genotype of the population that the stability of development or the genotype-environment interaction are altered, and they may affect the linkage relationships of relevant polygenes. Models will be presented that predict a decrease of genetic variance with stabilising selection and an increase with disruptive selection, on the assumption that the initial population contains some heterozygous polygenic complexes in the repulsion phase. Some disruptive selection models also result in a change of the mean value of the character. Two types of disruptive selection are considered. In one High and Low extremes are selected and mated together. In the other High is mated to High and Low to Low, and both High and Low are selected from the offspring of each. The two models give qualitatively similar expectations, though the second is more effective.

Experiments with sternopleural bristle number in *D. melanogaster* are giving results that agree very well with the expectations. Directional selection is proving very effective in a line previously exposed to disruptive selection, but has had no significant effect on a line previously exposed to stabilising selection. Its effect on the foundation stock from which the lines were derived has been intermediate.

CHROMATID ABERRATIONS INDUCED BY SUBSTITUTED ETHYLENEIMINES

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One polyfunctional and two monofunctional ethyleneimines inhibit growth of the Walker carcinoma in the rat to varying degrees.

Analysis of the different cytotoxic effects induced in *Vicia* roots by the same compounds showed that chromosome breakage is the only one of these effects which is closely correlated with the degree of tumour inhibition. It appears that, for ethyleneimines to be effective in inhibiting tumour growth, they must induce an adequate level of chromosome breakage, at concentrations considerably below those which result in cell lethal effects.

Chromatid aberrations in *Vicia* root-tip cells are induced by the action of ethyleneimines during the early resting stage. Heterochromatic regions of the chromosomes and homologous chromosomes are preferentially involved in the aberrations. Ford and Revell have previously reported the same non-random distribution of aberrations for nitrogen mustard and a di-epoxide. As the time interval between treatment and sampling is increased, the ratio of chromatid exchanges *between* chromosomes to those *within* a chromosome increases, while the proportion of aberrations involving the long, nucleolar chromosome also increases. These results will be interpreted in terms of Revell's chromatid exchange hypothesis.

ENZYME PRODUCTION BY MUTANT ALLELES AT THE *AM* LOCUS IN *NEUROSPORA CRASSA*

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and
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Several independently induced mutations are known at the *am* locus in *Neurospora*, each resulting in the loss of the ability to produce detectable glutamic acid dehydrogenase. The work reported here is concerned with three of these mutants, designated am^{29}, am^{32} and am^{47}. A few wild types have been obtained from $am^{29} \times am^{47}$ and $am^{32} \times am^{47}$ crosses with a frequency of about 0·001-0·003 per cent. These wild types breed true and possess enzyme activity comparable with the stock wild types. In both $am^{29} \times am^{32}$ and $am^{32} \times am^{47}$ crosses, pseudowilds are recovered with frequencies of about 0·2 and 0·05 per cent. respectively. The pseudowild strains do not carry a wild type allele but apparently originate as disomics carrying two *am* alleles and they subsequently break down into heterocaryons. Both pseudowilds and heterocaryons obtained by vegetative combination of am^{32} and am^{47} strains possess 15-50 per cent. of the usual enzyme activity of the wild type. Thus different *am* alleles are complementary in action with respect to glutamic dehydrogenase production when the two alleles are carried in separate nuclei in a heterocaryon. No pseudowilds have been obtained from am^{29} and am^{32} crosses. It seems that this is a case of a complex locus closely concerned with the production of a specific enzyme.

A QUALITATIVE DIFFERENCE BETWEEN ALLELES AT THE *AM* LOCUS IN *NEUROSPORA CRASSA*

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A new allele am^{1} was obtained by ultraviolet-induced mutation in an am^{47} strain. Strains carrying am^{1} grow almost as well as wild type on minimal medium at 25° but show a marked requirement for exogenous amino nitrogen at 20° or lower temperatures. They produce a glutamic dehydrogenase which differs from that produced by the wild type in that it has very little activity as extracted at 20° or lower but is capable of being activated by mild heat treatment or, to some extent, by incubation with substrates. The heat activation is reversible ; the extra activity is slowly lost on returning the enzyme to room temperature but can be regained on repeated heat treatment. Wild type and am^{1}-type glutamic dehydrogenases have both been considerably purified without obvious alteration in their respective properties. The two types of enzyme appear to show no interaction in mixtures. Experiments on heterocaryons show evidence of complementary interaction between am^{1} and am^{32}, but none between am^{1} and am^{47}.

GENETIC STUDIES WITH *SALMONELLA TYPHIMURIUM*

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A review will be given of the most important results obtained in our laboratory in research with *S. typhimurium*. The review will deal with the mechanism of transduction ; the genetically complex structure of gene loci ; tests for allelism by means of abortive transduction ; linkage relationships ; the limitations of the transduction method for detection of linkage ; the non-random distribution of genes on bacterial chromosomes ; and the action of suppressor mutations.

HISTIDINE-REQUIRING MUTANTS OF *SALMONELLA TYPHIMURIUM*: COMPLETE AND ABORTIVE TRANSDUCTION

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The mutational sites of 44 histidineless mutants are localised within a small chromosome region. The region is divisible into five functional units on the basis of biochemical characterisations of mutant phenotypes. Each unit is further divisible by mutation and recombination. The order of the units on a linkage map parallels precisely the sequence of the biochemical steps affected : (1) formation of imidazoleglycerol phosphate ester (IGP), (2) IGP dehydrase (locus B), (3) imidazole-acetol phosphate ester transaminase (locus C), (4) L-histidinol phosphate ester phosphatase (locus G), and (5) L-histidinol dehydrogenase (locus D).

Results of over 400 different mutant combinations, tested for functional complementariness in abortive transduction, confirm the above locus designations. Further, sites of mutation in the first unit (IGP formation) are divided into three complementary classes (loci E, F and A). All of the mutational sites are confined to single loci and do not inactivate the functioning of adjacent loci. The ratio of abortive to complete transductions (usually 10/1 with phage grown on wild-type cells in nutrient broth) may rise to 250/1 in tests between complementary mutants ; additional recovery of the mutant donortype returns the ratio to 10/1. Both as donor and as recipient, *hi*-32, located at an extreme end of locus B, is exceptional in showing complementariness with some other mutants of its own locus. This may be due to the unique " leaky " nature of *hi*-32 and a gene-dosage effect, enhancing reactions on either side of the genetic block.

RELATIONAL INCOMPATIBILITY IN DIPLOID AND TETRAPLOID LUCERNE

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In both *Medicago coerulea* Less. ($2n = 16$) and *M. sativa* L. ($2n = 32$), the more inbred the zygotes to be produced by a mating, the less fertile is the mating. In both diploid and tetraploid, if fertility is expressed as pods per flower pollinated, then each increase of 0·01 in the inbreeding coefficient of the offspring gives a reduction of just over 0·01 in the fertility of the mating. As this type of incompatibility depends on the relation between uniting alleles, it is proposed to call it relational incompatibility. It has interesting implications with reference to the use of inbred lines in lucerne breeding and to the evolution of self-incompatibility.

THE COST OF EVOLUTION

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Natural selection means that members of genotypes ill-adapted to the environment die prematurely or are partly sterile. The unit process of evolution is the replacement of one gene by another in a population. The total number of deaths involved in this process is independent of the intensity of selection when this is small. It depends on the rarity, before selection starts, of the gene selected, and on the degree of its dominance. A representative number of deaths is 30 times the number of individuals in a generation. Thus evolution at the rate of one gene substitution in 300 generations would lower the reproductive capacity of a species by about 10 per cent. This fact sets an upper limit to the rate of evolution.

INHERITANCE OF MILDEW RESISTANCE IN *FRAGARIA* WITH SPECIAL REFERENCE TO CYTOPLASMIC EFFECTS

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The incidence of mildew varied from light to very heavy in a large range of commercial octoploid types (*Fragaria grandiflora*). The diploid group (*Fragaria vesca* L.) was in general less severely attacked, though examples occurred of extreme susceptibility. A haploid *vesca* was completely immune but the corresponding diploid was lightly attacked. In a cross of susceptible × relatively resistant, susceptibility appeared to depend on two major genes. Evidence was found of cytoplasmic effects on mildew manifestation. The grade of susceptibility differed in reciprocal crosses. Cytoplasmic influences appeared to persist in the F_2 and back cross generations.

MEIOSIS IN *SPIROGYRA*

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Meiosis in *Spirogyra* has been found for the first time since the only two records by Karsten, 1908, and Trondle, 1911 : for the first time in the species *crassa*. The first meiotic division is equational, sister chromatids separating, non-sister chromatids remaining associated in pairs, as in *Luzula* and other organisms without a localised centromere. Three of the four products of meiosis abort. Strains derived from zygospores are being maintained in culture.

ACCELERATED RESPONSE IN SELECTION EXPERIMENTS

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Selection lines, which, after steady response to selection for some generations, go through a period of relatively rapid response, are of considerable interest in relation to the limitations to which response to selection is subject. Two related lines of *D. melanogaster*, both selected for high sternopleural number, have responded in very similar manner, rising in 30 generations from 19 chaetæ to nearly 30 chaetæ, with a very rapid response from about 24 to about 28 chaetæ in 5 generations. The similar behaviour of the two lines makes mutation an unlikely explanation of the accelerated response. Alternative hypotheses are (1) that there are loci heterozygous for alleles neutral in their effects on bristle number in some genetic backgrounds, but exploitable by selection after the genetic background has been changed and (2) that the accelerated response occurs after rare recombinants have been formed, perhaps when the gene complexes have been changed so that such recombinants become more likely.

Preliminary experiments suggest that the 4 bristle difference involved in the accelerated response in our lines is associated with a chromosome region whose effects can be detected on a background giving a mean below 24 bristles. This result does not support hypothesis one. The effect seems to be associated with a region of chromosome III close to or in the heterochromatin.

NUCLEAR DIVISION IN THE EUGLENINEÆ

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Nuclear division has been observed in 18 species of the Euglenineæ, representing 11 genera. Evidence has been obtained on several major points of controversy,

in particular that the chromosome cleavage is longitudinal and not transverse as has been maintained by a large body of workers. Nuclear division occurs almost exclusively by a very peculiar form of mitosis, the main features of which are (*a*) the persistence of the endosome (" nucleolus ") as a dividing body, (*b*) the apparent complete absence of centromeres, centrioles and spindle, (*c*) the arrangement of the chromosomes longitudinally to the division axis at metaphase, (*d*) the retention of the nuclear membrane throughout division. All species have a high chromosome number.

Very recently, the first conclusive evidence of a meiotic process has been obtained. It is probably associated with a form of autogamy. The details of the meiosis so far observed are unlike those recorded for other organisms.